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Polycyclic Aromatic Hydrocarbons: Evaluation of Sources and Effects

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Polycyclic Aromatic Hydrocarbons: Evaluation of Sources and Effects

Committee on Pyrene and Selected Analogues

Board on Toxicology and Environmental Health Hazards

Commission on Life Sciences

National Research Council

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The present report attempts to make current the information relative to the sources (both mobile and stationary), formation, atmospheric transformations, biologic effects, and pharmacokinetics of a select group of polycyclic aromatic hydrocarbons (PAHs) and mixtures thereof, to identify populations hypersensitive to them and to determine the human risks associated with exposure to them. The specific PAHs considered were chosen on the basis of relative concentrations in various kinds of emission or combustion products or because of some unique pharmacologic property.

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POLYCYCLIC AROMATIC HYDROCARBONS:
EVALUATION OF SOURCES AND EFFECTS

COMMITTEE ON PYRENE AND SELECTED ANALOGUES

BOARD ON TOXICOLOGY AND ENVIRONMENTAL HEALTH HAZARDS

COMMISSION ON LIFE SCIENCES

NATIONAL RESEARCH COUNCIL

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NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competence and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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This document is the result of individual and coordinated efforts by the members of the Committee on Pyrene and Selected Analogues. Although individual members were responsible for specific sections, the entire report was reviewed by the full Committee. The summary (Chapter 8) and the recommendations (Chapter 9) represent a consensus of the Committee members.

The executive summary was prepared by the chairman, Dr. Edward Bresnick. Chapters 1, 2, and 3, on sources and atmospheric transformations and persistence, represent a joint effort of Drs. Robert A. Gorse, Jr., Daniel Grosjean, and Ronald A. Hites and Mr. James A. Frazier. Chapter 4, on biologic effects, was written by Dr. Bresnick. Chapter 5, on pharmacokinetics and effective biologic dose, was prepared by Drs. Marshall W. Anderson and James K. Selkirk. Chapter 6, concerning human exposure to and metabolism of the compounds in question, was written by Dr. Attallah Kappas. Chapter 7, on populations of "hypersensitive" persons, was written by Dr. Richard E. Kouri. Appendix C, dealing with human-cancer risk assessment, was prepared by Dr. Malcolm C. Pike. Appendix D, on public decision-making with respect to source and emission control, was prepared by Dr. Lawrence J. White.

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EXECUTIVE SUMMARY

The Clean Air Act stipulates that from time to time the Administrator of the Environmental Protection Agency (EPA) shall revise a list that includes pollutants that may be anticipated to endanger public health or welfare and for which air-quality criteria have not been issued.

As part of a continuing contract with the National Academy of Sciences to prepare scientific and technical assessment reports on selected pollutants, the EPA asked for an evaluation of selected and representative pyrene compounds and their analogues as they occur as pollutants in the ambient air, especially those from mobile sources.

The Committee on Pyrene and Selected Analogues, appointed by the National Research Council, selected representative pyrenes and close chemical relatives for study. Great difficulties necessarily are encountered when a study covers a large number of compounds. It is extremely difficult to be comprehensive and discuss every compound in detail. The Committee found that there were far more sources of human exposure to pyrenes than vehicle exhaust--for instance, cigarette-smoking, coke ovens, wood-burning, and some foods. The Committee is aware that some of its interpretations are founded on data that are neither clear-cut nor complete. This is true of its efforts to extrapolate risks, to identify susceptible groups in the population, and to assess economic alternatives for control or abatement of the pollutants in question.

The polycyclic aromatic hydrocarbons (PAHs) have been reviewed previously as components of atmospheric pollution and as potential human-health hazards. This document attempts to make current the information on the sources, formation, atmospheric persistence and transformations, biologic effects, and toxicokinetics of a select group of PAHs and on the identification of populations hypersensitive to them. The document also presents material on human risk assessment and develops an approximate estimate of the societal value of reducing environmental emission of benzo[a]pyrene. Benzo[a]pyrene is used as a surrogate PAH. It may not be the best indicator of the biologic effects of other PAHs in soots and smokes. However, the literature on benzo[a]pyrene is considerably more voluminous than that on other PAHs. It should also be recognized that the benzo[a]pyrene concentrations in soots and smokes is small and that other PAHs present in smokes have greater biologic activity, such as nitro-PAHs. The specific PAHs discussed in this report were selected on the basis of their relative concentrations in various emission or combustion products or because they are pharmacologically active. The structures of the selected compounds are presented in Appendix A.

SOURCES, ATMOSPHERIC PERSISTENCE, AND TRANSFORMATIONS OF PAHs

The total annual release of PAHs from mobile sources in the United States has been estimated. In the case of benzo[a]pyrene (BaP), all mobile sources produced about 43 metric tons in 1979, including 27 tons from motor vehicles; 63% and 37% of the BaP emission from motor vehicles occurred in urban and rural areas, respectively. It is projected that the total motor-vehicle BaP emission will decrease by the year 2000 to 24 metric tons, of which only about 40% will be in urban regions. This implies a shift toward emission in the rural areas. This projection is based on the adoption of no further changes in emission standards beyond those which have been in effect since 1980. If more rigorous emission standards are adopted in 1985 or later, BaP emission could be considerably lower.

On the basis of motor-vehicle emission values for 1979, the average daily BaP concentration in the urban atmosphere has been calculated as 1.3 ng/m^3; this value is in excellent agreement with the findings in Los Angeles, where atmospheric BaP comes largely from motor-vehicle exhaust. Lower-density urban areas would have lower atmospheric concentrations of motor-vehicle-contributed BaP. In the year 2000, atmospheric BaP concentrations in cities with the traffic conditions of Los Angeles are expected to be approximately 0.6 ng/m^3--a large decrease from 1979. So-called "hot spots" of BaP, as in severe roadway tunnel congestion, can lead to motor-vehicle-generated BaP concentrations of approximately 50 ng/m^3, to which people would be exposed for very brief periods. Atmospheric BaP concentrations as high as 74 ng/m^3 have been measured in U.S. urban areas (such as Birmingham, Alabama); much of this BaP is contributed by stationary sources.

The high atmospheric concentrations arise mostly from stationary combustion, especially that of coal, wood, and oil. National annual BaP atmospheric emission from all combustion sources, including both mobile and stationary, is estimated at between 300 and 1,300 metric tons. The total is decreasing because of emission controls and changes in fuel use--e.g., less coal is used in residential furnaces, but more coal is used in power generation. Wood-burning stoves and fireplaces, currently nonregulated sources of PAHs, are ubiquitous and are important and increasing sources of atmospheric PAHs in urban areas, as well as in rural areas with restricted air flow.

A survey of the literature reveals large uncertainties with respect to the persistence of PAHs, their chemical transformations, and their transport and fate in the atmosphere. There is evidence from long-range transport studies and analyses of sediments that PAHs may be transported over long distances in the atmosphere (e.g., 1,000 km) without substantial degradation; and laboratory studies have shown that many PAHs react chemically with atmospheric components in a matter of hours.

High chemical reactivity and long-range transport of unreacted PAHs are not necessarily in disagreement. There is a need for more information on this extremely broad and dynamic issue. The major chemical processes include photooxidation, reaction with ozone, and reaction with nitrogen dioxide--the latter can yield potent, direct-acting mutagenic nitro derivatives. However, these processes appear to be significantly slower for PAHs adsorbed on atmospherically relevant substrates, such as soot and fly ash, than for the same PAHs deposited on filters in pure form. It is still uncertain whether nitro-PAHs occur in exhaust or are artifacts of the filter-sampling procedures.

BIOLOGIC EFFECTS

Particles from diesel engines were tested for toxicity in intact animals. Only minimal effects on pulmonary function, reproductive capacity, glandular or hepatic function, and general neonatal health were observed. There was some indication of abnormal development of central nervous system function in newborn rats that were chronically exposed to diesel-engine exhaust. There was not enough information to ascribe these alterations to the PAH content of the exhaust.

Organic-solvent extracts of particles from spark-ignition-engine and diesel-engine exhaust were mutagenic in *Salmonella typhimurium* in forward- and backward-mutation assays and were mutagenic in several animal-cell model systems. In bacterial assays, diesel and spark-ignition combustion products were directly active; emission from coke ovens and roofing tar, cigarette-smoke condensate, wood combustion products, and the positive control BaP required metabolic activation before they demonstrated any mutagenic action. The mutagenic efficacy of the combustion products was reduced by the inclusion of alcohol in the fuel base (a suggestion of additional advantage to its presence in fuel). The direct-acting mutagenic property appeared to be caused in part by nitro-PAHs. The latter were constituents of the automobile-exhaust particles, but were not found in wood combustion products. The nitro-PAHs tested proved much more active mutagenically than the parent compounds. Indeed, 1,8-dinitropyrene, a constituent of particles and, in the past, of xerographic toners, was the most highly mutagenic of all compounds subjected to the Salmonella/microsome assay. The nitro-PAHs have not been tested consistently in animal-cell mutagenesis models or whole animals, however. Because of their mutagenic potency observed in the Salmonella/microsome assay, it is essential to learn whether they are formed in exhaust products or are artifacts produced during the course of the sampling procedure.

The extracts of particles from mobile and stationary sources have been tested for carcinogenicity, mainly by topical application to mouse skin. The condensates from spark-ignition-engine exhaust were carcinogenic in this model, and diesel-exhaust preparations were less active. The exhaust preparations exhibited both initiation and promotion activities in the skin-tumor model. Whether the tumorigenicity reflected the additive activity of a number of PAHs present as components of the condensates depended on the experimental protocol used. The cocarcinogenic activity of mixtures of PAHs must be studied further and the activity relationship of individual chemicals measured. The compounds of the condensates were not very active in the inhalation or intratracheal-instillation test systems used for tumorigenicity, and the major routes of entry of emission and of PAHs are ingestion and inhalation. Additional effort should be expended to develop test models that better approximate human carcinogenesis.

The nitro-PAHs have not been tested for tumorigenicity. Because they are potent mutagens, they should be tested in several tumorigenicity model systems. Although there was a moderate correlation between the mutagenicity and the carcinogenicity of the PAHs, a battery of tests--including assays for mutation, clastogenesis, primary DNA damage, and morphologic transformation--would serve better as a monitoring mechanism.

EFFECTIVE BIOLOGIC DOSE

PAHs are readily absorbed after administration to laboratory animals by various routes and are distributed to a number of tissues. Nonmetabolized PAHs accumulate and persist in fat to a much greater extent than in other tissues. This phenomenon may be used to monitor the chronic exposure of populations to sources of PAHs. PAHs that are present on particles are retained in the lungs of animals to various degrees as a function of particle size and composition. The particle-bound PAHs are desorbed in the lung and distributed systemically to various tissues.

The clearance of PAHs from animals is a function of the "reservoir" of nonmetabolized material in the fat, metabolism, biliary excretion, fecal excretion, and, to a smaller degree, urinary excretion. The excreted metabolites consist of glucuronides, sulfates, and unconjugated hydroxylated and phenol derivatives.

The metabolism of many of the PAHs has been studied in in vitro systems. Preparations from virtually all tissues are able to metabolize PAHs, although liver is the most efficacious in this regard. The initial metabolism is catalyzed by membrane-bound cytochrome P-450-dependent monooxygenases. The epoxide product may spontaneously rearrange to a phenol, which may give rise to conjugated phenols. The epoxides serve as substrates for another membrane-bound enzyme, epoxide hydratase, which catalyzes the formation of trans diol derivatives; these may also be excreted in conjugated form (as glucuronides) or in unconjugated form.

The activation of PAHs to at least some of the ultimate carcinogenic forms requires recycling of the trans diol derivatives through the cytochrome P-450-dependent monooxygenases to yield very reactive diol-epoxides, which can spontaneously form electrophiles capable of interacting with macromolecular components, such as DNA. The amounts of monooxygenases and epoxide hydratase in tissues are genetically determined; and the enzymes are inducible. Indeed, PAH exposure can increase dramatically the amount of monooxygenase; the extent of induction is also genetically determined.

The activation reaction can also be fulfilled through an arachidonic acid-dependent cooxygenation step involving prostaglandin peroxidase. In this step, the trans diol is enzymatically transformed to the diol-epoxide at the expense of prostaglandin G_2. Hence, prostaglandin synthesis may be intimately involved in the elaboration of carcinogenic agents from PAHs.

PAH metabolites, such as diol-epoxides, interact in covalent fashion with DNA bases to form adducts. The adducts of BaP diol-epoxide with DNA--BP DE-DNA adducts--form readily in lung, liver, forestomach (of mice), colon, kidney, brain, and muscle after oral administration of the PAH to laboratory animals. Human tissues also have this capacity. From in vivo studies, the BP DE-DNA adduct profiles appear representative for a particular tissue. The major adduct known to be formed is between BP DE I and the 2-position of deoxyguanosine in DNA, and a minor adduct is formed at the 6-position of deoxyadenosine. The adduct profile is species-dependent. The quantitative aspects of these reactions do not appear to be correlated with the susceptibility of a tissue to PAH-induced carcinogenesis. For example, hepatic tissue is not under ordinary circumstances a target organ for PAHs, but it can easily biosynthesize these adducts.

The PAH-DNA adducts have various turnover rates in different tissues. The relative contributions of a hitherto unknown DNA enzymatic repair system and cell turnover have not been established under in vivo conditions. It is apparent, however, that different adducts are removed from the DNA at different rates.

A linear dose-response relationship has been observed (with BaP) for PAH metabolite-DNA adducts even at low doses. There appears to be no threshold dose below which binding of activated PAH metabolites to DNA does not occur. The administration of several inducers of the cytochrome P-450-dependent monooxygenases and of various conjugating enzyme systems, as well as the administration of several antioxidants, dramatically reduces the formation in vivo of adducts. That suggests that these substances (i.e., antioxidants and monooxygenase inducers) may decrease PAH induction of neoplasia as a result of their ability to affect synthesis of adducts. It is proposed that, given a "susceptible" tissue (i.e., one that is neoplastically transformed by a PAH), the adduct concentration can be an appropriate measure of the "effective biologic dose" of the PAH. With current sensitive radioimmunoassay methods at our disposal for the determination of these adducts, it should be relatively easy to determine that concentration, e.g., in human lymphocytes.

The formation of adducts may have three important implications for toxic effects in humans: (1) Even at low environmental concentrations of a PAH, continuous exposure could result in persistent formation of the adducts, leading to a higher incidence of neoplasia. The persistence of the adduct in a particular tissue will be determined by the extent of repair or cell turnover. (2) The presence of an adduct, even at a low concentration, may influence replication, transcription, and transposition substantially. In any case, the expression of the genome will be affected. The extent of this problem will depend on the site of PAH-DNA adduct formation. (3) Nutrition and other exogenous factors may influence the activation of a PAH, the extent of conjugation to a detoxified product, the formation of adducts, and the relative turnover of these adducts in a particular tissue.

HUMAN EXPOSURE TO AND METABOLISM OF PAHs

Human exposure to PAHs is almost exclusively via the gastrointestinal and respiratory tracts--and approximately 99% of these substances is ingested in the diet. PAHs are ubiquitous in foodstuffs. The PAH content of most foods before preparation is quite low, but some have surprisingly high concentrations, presumably as a result of pollution from soils, irrigation waters, and atmospheric fallout and perhaps from the initial phases of food-processing. The contaminants include 100 or even more PAHs. The mode of cooking, especially broiling, also affects the composition and quantity of PAHs in foods.

The extent to which PAHs gain access to the circulation is not known. Both cellular and humoral routes of entry appear to be involved. The serum lipoproteins constitute a substantial circulatory pool of PAHs. PAHs presumably translocate from these serum proteins into cells by a non-receptor-mediated mechanism. According to data obtained with lower animals, the PAHs will enter cells or accumulate in fatty tissues and then slowly re-enter the circulation, undergo a variety of biotransformations, and are excreted via the biliary or the urinary system. There is a dearth of information on the human toxicokinetics of PAHs other than BaP.

Human normal and malignant tissues have the metabolic capacity to effect oxidative transformations of PAHs, especially BaP, to form products--including ultimate carcinogens--comparable with those formed in experimental animals. There is a very large individual variation in these enzymatic activities, and the rate of oxidative metabolism of a PAH can vary considerably in different sites in the same organ from the same organism. The PAHs are present in various human tissues to a limited extent, and some can be sporadically, or even regularly, identified. It is important to note this observation, although the relation of the finding of specific pathologic conditions to known PAH pollution has not been established. Nor has a biochemical "marker" involving PAHs been established by which a patient population with specific enzymatic characteristics can be distinguished in relation to a discrete pathologic condition. The question of high inducibility of aryl hydrocarbon hydroxylase (AHH) activity in lymphocytes and monocytes of lung-cancer patients continues to be provocative, as well as ambiguous, and the use of this activity as a marker of high-risk populations deserves further study.

There is very little information to implicate diet-derived PAHs in any form of clinical pathologic condition, despite the high concentrations of these compounds to which humans may be exposed through food contamination. The lack of information suggests that the gastrointestinal system (including the liver) may be relatively "resistant" to the toxicity of PAHS or that this system can biochemically adapt to PAH exposure. The capacity of the body's detoxification system to be "resistant" is worth exploring.

POPULATIONS OF HYPERSENSITIVE PERSONS

The toxicity--including mutagenesis, carcinogenesis, and teratogenesis--of PAHs results from multistage processes, and variations in any of the intermediate stages can influence susceptibility to the effects. Sensitivity to PAH-induced biologic effects is probably controlled at the level of uptake into particular cells, metabolic activation or inactivation of the parent PAH chemical, capacity of cells to repair PAH metabolite-DNA adducts, capacity of cells to express DNA damage and allow progression to the phenotype of a mutant or tumor cell, and immunocompetence of the host. Compilation of data from humans and animal-model systems has demonstrated a degree of genetic regulation of each of these stages, but the information is far too sketchy for specific conclusions to be drawn on the role of PAHs.

PAHs in both human and animal systems are taken up and metabolized by microsomal monooxygenases that are under some sort of genetic regulation. In murine-model systems, susceptibility to carcinogenesis induced by PAHs is genetically linked to the capacity to respond to and metabolize these chemicals. In humans, development of cigarette-smoke-associated lung cancers also may be linked to the capacity to respond to and metabolize PAHs. Natural variations in DNA-repair capacity do occur among humans with specific genetic disorders, and these persons are more susceptible to cancer; but whether PAHs play a role in such susceptibility is not known. Variations in capacity to promote (or allow for progression of) carcinogenesis occur in animal-model systems, but there are virtually no data or similar variations among humans. Genetically controlled variations in immunocompetence are observed in humans, and persons with these alterations are usually more susceptible to carcinogenesis; but, again, no active role for PAHs has been suggested. This lack of information on the impact of genetic variation is striking. Factors that tend to make these genetic differences less distinct include the physical state of the PAHs and the nutritional and developmental states of the host.

DISCUSSIONS OF RISK ASSESSMENT AND PUBLIC-POLICY COST-BENEFIT CONSIDERATIONS

The Committee draws conclusions about neither anticipated health risks associated with exposure to PAHs in ambient air nor the societal costs of reducing PAH emission. However, some examples of how these considerations could be addressed are described in Appendixes C and D, respectively.

INTRODUCTION

Benzo[a]pyrene is a chemical commonly found in the emission products from most types of fuel combustion, whether it occurs in the engine of an automobile, the fireplace of a home, or an industrial installation. It has been found to be one of the chemicals that cause cancer in humans exposed to them. Because this chemical and other PAH compounds emitted in automobile exhaust are commonly found in the ambient air (see lists in Appendixes A and B), the U.S. Environmental Protection Agency asked the National Academy of Sciences-National Research Council to assess the health risks of humans exposed to the compounds that can be identified and characterized in the atmosphere, to identify those persons most susceptible to the toxic effects of the compounds, and to characterize the other major sources of human exposure, with emphasis on emission from mobile sources. The NRC Committee on Pyrene and Selected Analogues, which prepared this report, was formed in the Commission of Life Sciences, where it was under the oversight of the Board on Toxicology and Environmental Health Hazards.

The relatively recent oil crisis in the United States has focused attention on efforts to reduce the amount of crude oil that must be imported to maintain our standard of living. As part of the move to conserve fuels, there has been an increase in the use of diesel engines. Unfortunately, diesel-engine exhaust contains more particulate matter than exhaust from spark-ignition engines and therefore may contribute heavily to environmental pollution. The major organic chemical constituents attached to the particulate matter in diesel exhaust include the polycyclic aromatic hydrocarbons (PAHs). Benzo[a]pyrene is commonly used in the literature as a surrogate for the whole class of PAHs, although it may not be the best indicator for the biologic effects of complex chemical mixtures containing PAHs. (See discussions of tracer chemicals and surrogates in concluding section of Chapter 3.)

One of the major objectives of the Committee was to evaluate the relative contributions of the various emission sources--mobile and stationary--to the PAH pollution burden and to establish the health risks to those exposed to the emission. The Committee selected the following compounds and close chemical relatives as the subjects of published information to use in preparing its report: acridine, benz[a]anthracene, benzo[a]pyrene, benzo[e]pyrene, chrysene, coronene, cyclopenta[cd]pyrene, dibenzothiophene, 1-fluoranthene, fluorenone, methylfluorenone, nitropyrene, phenanthrene, phenanthrene carboxaldehyde, and pyrene. (See Appendix A for formulas and CAS numbers of these and other PAHs.) The term "polycyclic aromatic hydrocarbon" (PAH) has been used throughout the report to refer broadly to all these chemicals.

The Committee met for the first time in May 1981. In its review of the published literature then being assembled, it became clear that PAHs have numerous sources and are ubiquitous in the environment. For example, the published information revealed that the human diet was a major source of exposure. Thus, if the adverse human health effects attributable to exposure to PAHs in the emission of automobiles and other vehicles were to be appropriately assessed, the Committee felt that information on exposure associated with other than mobile sources was needed, so that the relative amounts from each could be described. The Committee discussed mechanisms and principles for identifying population subgroups that seem to be more susceptible to the effects of exposure to PAHs. They discussed the mechanisms of chemical intoxication, the metabolism of individual PAHs, and the formation of adducts to DNA.

Chapters 1-3 of the report discuss the mobile and stationary sources of PAHs emitted to the atmosphere, their atmospheric persistence and transformations, and their deposition. Chapter 4 gives an overview of published findings on PAH toxicity and biologic effects. Chapter 5 describes the pharmacokinetics of PAH and their role in the formation of DNA adducts. Chapter 6 discusses human PAH metabolism, the modes and extent of human exposure to PAHs in the diet (via various foodstuffs and cooking methods) and from other sources, and deposition in various body tissues.

Chapter 7 discusses enzyme systems and genetic and other anomalies that can be used to identify or characterize persons who are hypersensitive to PAHs.

Chapter 8 summarizes the Committee's findings. In Chapter 9, the Committee presents recommendations for research that it feels will advance the understanding of the effects of PAH.

In trying to assess the adverse health effects, the Committee discovered a lack of epidemiologic data on exposure to PAHs from mobile or stationary sources and effects directly attributable to them. Perhaps the best epidemiologic work was related to cigarette-smoking, and smoking was therefore used as a model in the discussions of risk in Appendix C. However unsatisfactory this may appear at first glance, cigarette smoke is a complex mixture of PAHs and other chemicals, and the results provide some foundation for judging the relation of PAH exposure to adverse health effects observed in cigarette-smokers. The lack of data made it difficult (and perhaps it is impossible) to characterize cost-benefit relations with respect to the control or abatement of PAH emission from various sources and the attendant policy choices (see Appendix D). The cutoff date for literature cited in the report was June 1982.

Medium-duty trucks (gross vehicle weight, 8,500-33,000 lb) are now equipped with spark-ignition engines, but are also undergoing dieselization rapidly. Heavy-duty trucks (>33,000 lb) are already more than 90% diesels. In 1980, about 15% of new trucks sold were diesel-engine vehicles, and this figure could grow to 50% by the year 2000. In addition, the total number of trucks in the United States is expected to increase by 5% per year until beyond 2000.[44,46,60]

Approximately 90% of all commercial buses are powered by diesel engines; school buses are still powered by spark-ignition engines. Trains and ships are powered by diesel engines, as is most industrial equipment. Private boats and planes are powered predominantly by spark-ignition engines. Commercial aircraft are powered by gas-turbine engines that use jet fuel.

Although information on engine types used in military vehicles is not readily available, there is some information in Table 1-3 on military fuel consumption. Emission from military vehicles is not a major source of atmospheric PAHs, except possibly in particular areas.

PAH EMISSION FROM MOBILE SOURCES

Internal-combustion engines emit gases, liquids, and solids from the exhaust system as products of the incomplete combustion of the fuel and as noncombusted fuel, lubricants, and fuel additives. Chemical processes also occur in the exhaust system, especially in the catalytic emission-control devices. Some reactions continue after the exhaust is released into the atmosphere. The temperatures in the combustion chamber and in the exhaust system and the volume flow rates depend directly on engine design, size, operating speed, and working load. These factors are important in the formation of PAHs and in the amounts of PAHs that are emitted into the atmosphere.

The combustion process in a spark-ignition engine takes place with near-stoichiometric amounts of oxygen at temperatures in the vicinity of 3500°C. In diesel engines, there is an excess of oxygen with combustion temperatures in the vicinity of 2000°C. Exhaust temperatures for spark-ignition engines are commonly between 400 and 600°C, but diesel exhaust is typically at 200-400°C (except at high load factors). Oxidation catalysts typically must be at 400°C or higher before becoming active, so current types of oxidation or three-way catalysts do not function efficiently on diesel vehicles.[29,43]

Many of the PAHs have boiling points of 200-300°C and are sufficiently volatile to exist predominantly in the gas phase at temperatures above 200°C. Even at room temperature, some of the more volatile PAHs are distributed between the vapor and particle-adsorbed phases.[32]

In the open air, vehicular exhaust is diluted by a factor of about 1,000 in the first few seconds, so cooling to near-ambient temperature is quite rapid. But condensation of PAHs, by adsorption on existing particles, can occur many feet behind a vehicle, thus allowing some mixing of exhaust plumes from different emission sources. Normally, black "elemental carbon" particles,* also products of incomplete fuel combustion, act as condensation nuclei for the condensation of vapor-phase organic chemicals, such as aliphatic compounds, aromatic compounds (including the PAHs), aldehydes, ketones, acids, and heterocycles.

The exhaust is the major source of the PAHs; another possible source of PAHs is engine oil, because it can act as a sink for them. It has been estimated that crankcase oil collects 10 times as much PAH per mile traveled as is released from the exhaust system.[70] In the case of vehicles in which volatile emission from the crankcase is not controlled, it could be a significant source of PAHs in the atmosphere, but quantitative assessment is not now possible.

Studies of particle-size distribution of spark-ignition and diesel exhaust particulate material show mass-median aerodynamic diameters of 0.1-0.25 μm.[20,36,63] More than 90% of the mass is in particles less than 1 μm in diameter.[72] Larger particles presumably result from deposition of particulate material on and later release from the walls of the exhaust system. Resuspended road dust, roadbed material, and tire particles result in particle sizes of about 8 μm in median diameter, which can account for as much as 10% of the measured vehicular respirable particulate mass in near-road measurements.[68,72]

Spark-ignition vehicles with oxidation catalysts emit particulate material that is mostly aqueous sulfuric acid droplets with organic compounds presumably adsorbed on droplet surfaces. Particle median diameters tend to be somewhat less than 0.1 μm.[59]

Diesel particulate material is mostly elemental carbon. The primary particles are spherules 0.015-0.03 μm in diameter that agglomerate at high temperatures to irregular clusters and chains. These clusters, about 0.15 μm in diameter and containing up to 4,000 spherules, act as carriers of the PAHs and other adsorbed species.[86] The PAHs are adsorbed on the surface of the carbon and into the minute pores between the spherules. The small particle size results in long atmospheric residence times and in deposition in alveolar regions of the lung.[3,13,15,58,64] (See discussions in Chapter 3 on particles in the atmosphere and in Chapter 5 on relations of deposition of PAHs and particles.)

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*This material is not truly elemental carbon, nor is it graphitic carbon. No term has been found or accepted that properly describes the material.[92]

Exhaust-emission standards from EPA for HC, CO, and NO_x have resulted in 84, 79, and 56% reductions, respectively, in 50,000-mi emission from spark-ignition passenger cars, as shown in Table 1-4.[84] Particulate emission and lead emission have also decreased as a result of the use of catalysts and the decrease in lead concentrations in leaded fuel. Figure 1-2 shows the decrease in urban CO concentrations since 1973,[30] and Figure 1-3 shows the decrease in traffic-average lead emission rates as measured in highway tunnels.[72]

SAMPLING OF EXHAUST FROM MOBILE SOURCES

Techniques for sampling exhaust from mobile sources have been thoroughly described elsewhere and are not reviewed here except as pertinent to the analysis of PAHs.[4,21,53,77]

Exhaust-particle sampling in this country commonly involves the use of dilution tunnels. The dilution tunnel represents a laboratory attempt to simulate the normal atmospheric dilution and cooling of the exhaust. Atmospheric dilution is by about 1,000:1 in the first few seconds, whereas typical laboratory dilutions are between 5:1 and 20:1.[19,24] Exhaust from a vehicle tailpipe is mixed with particle-free, temperature- and humidity-controlled air in a tunnel that is typically 8-16 in. (20-40 cm) in diameter. Downstream from the exhaust inlet, a constant fraction of the diluted exhaust is pumped through a high-efficiency filter to collect exhaust particles. The weight gain of the filter is a measure of total particulate emission. Adsorbed organic matter, including PAHs, is isolated from the carbon particles by solvent extraction or other techniques. The organic extract material can then be analysed in many ways, including high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS or GC/MS), and bioassays.[5,42,53,77,79] The Salmonella assay has become a commonly used test in almost all laboratories working with vehicle-exhaust particulate material.

A technique applied more commonly in Europe uses low-temperature condensers (as many as three in series, at successively lower temperatures) followed by filtration.[33,34,35] This approach is used either on the undiluted exhaust or on the exhaust from a dilution tunnel. As mentioned previously, PAHs with high volatility (molecular weight <250) can be distributed in the vapor and condensed phases. Therefore, filter-only sampling from a dilution tunnel misses some of the more volatile PAHs. By combining the PAHs collected in each of the condensers with the PAHs collected on the filter, one obtains a better quantitative assessment of PAH emission. Results from filter-only dilution-tunnel studies are used to provide a qualitative description of PAHs in exhaust, and condenser-study results are used to provide quantitative emission rates.

In any attempt to sample a chemical system, it is necessary to show that the sampling process does not alter the chemical concentrations of the mixture. The production and destruction of chemicals during sampling are called "artifacts" of the sampling process. Sampling artifacts are important in a discussion of the production of the nitro-PAHs.

QUALITATIVE DESCRIPTION OF EMISSION FROM MOBILE SOURCES

The combustion of gasoline or diesel fuel in air yields water and carbon dioxide as the principal combustion products. Nitrogen oxides result from the high-temperature reaction of nitrogen in the air and from combustion of nitrogen-containing compounds in the fuel and lubricant.[1,41] Carbon monoxide, gas-phase hydrocarbons, elemental carbon, and particle-adsorbed organic material are formed as products of the incomplete combustion process. Fuel and lubricant additives and impurities and their combustion products are also found in exhaust. For example, sulfur-containing organic compounds in the fuel are combusted to gaseous sulfur dioxide, some of which can be further oxidized to sulfuric acid in the combustion chamber or in the oxidation catalyst and give rise to sulfuric acid in the particulate material.

The components detected as gas-phase hydrocarbons are listed in Table 1-5 (from a study of on-road gaseous organic-compound emission). The quantitative emission rates have not been determined.[37,38]

Diesel-exhaust particulate material has been the subject of extensive study in the last 5 yr. It is typically about 25% extractable into organic solvents, although different vehicles may have extractable fractions of 10-90%, depending to some extent on operating conditions. More than half the extractable material is aliphatic hydrocarbons of 14-35 carbon atoms and alkyl-substituted benzenes and naphthalenes.[4,42,78] The remaining extractable mass is PAHs and oxidized derivatives of the PAHs, such as ketones, carboxaldehydes, acid anhydrides, hydroxy compounds, quinones, nitrates, and carboxylic acids. There are also heterocyclic compounds containing sulfur, nitrogen, and oxygen atoms within the aromatic ring. The alkyl-substituted PAHs and PAH derivatives tend to be more abundant than the parent PAH compounds.

The particulate-extract HPLC eluent can be separated into nonpolar, moderately polar, and highly polar fractions. The fractions can then be further analyzed by GC/MS. Table 1-6 lists the results of such an analysis of the nonpolar and moderately polar fractions of a particulate extract from an Oldsmobile diesel vehicle, including the approximate extract concentrations for this particular vehicle.[78] The highly polar fraction has not been fully characterized. It contains the PAH carboxylic acids, acid anhydrides, and probably sulfonates and other highly polar species.[78,94]

Most (75%) of the direct bacterial mutagenicity resides in the moderately polar fraction (see discussions of Salmonella strains in Chapter 4).[10,71,73,74,76,80,81,88] The remaining direct mutagenicity is in the highly polar fraction. These aspects are discussed further in Chapter 4.

Over 50 chromatographic peaks of nitro-PAH compounds have been identified in diesel particulate extracts, as listed in Table 1-7.[22,40,47,65,75,93] 1-Nitropyrene is the most abundant of the nitro-PAHs, ranging from 25 to 2,000 ppm in the vehicle extracts studied. The other nitro-PAHs are present at concentrations from below the parts-per-million range to a few parts per million. The nitropyrenes have been studied in greater detail. They are released in diesel and gasoline exhaust (according to particulate extracts) at approximately 8.0 and 0.30 μg/mi, respectively. The latter value was obtained with leaded gasoline; with unleaded fuel, the rate was 0.20 μg/mi.[30]

Gibson[26] has determined the concentration of 1-nitropyrene in ambient particulate extracts obtained from suburban areas in Michigan to be 0.016-0.030 ng/m^3 of air (corresponding to 0.2-0.6 ng/mg of particles. Gibson has also observed that catalytic converters greatly reduce the concentration of nitropyrenes. The nitropyrene concentration in extracts from particles obtained from the emission of a wood-burning fireplace was less than 0.1 ng/mg of particles.[26]

1-Nitropyrene has been the only nitro-PAH detected in spark-ignition particulate extracts.[31,95] On-road heavy-duty diesel and light-duty spark-ignition vehicles have recently been found to have very low 1-nitropyrene particulate extract concentrations, which thus account for very small fractions of the on-road direct bacterial mutagenicity associated with these vehicle categories.[31]

QUANTITATIVE DESCRIPTION OF PAH EMISSION FROM MOBILE SOURCES

The work of Grimmer and co-workers,[33,34] of Kraft and Lies,[48] and more recently of Zweidinger and colleagues[31,95] can be used to derive typical rates of emission of many of the PAHs from the different categories of mobile sources. Because a relatively small number of vehicles have been used to measure these emission rates, the uncertainty in the derived vehicle-category emission factors is quite large--probably at least a factor of 2 and possibly even larger. Table 1-8 lists the best current measurements of rates of emission of numerous PAHs and their derivatives for spark-ignition vehicles (light-duty with and without catalysts and heavy-duty) and for light-duty and heavy-duty diesel vehicles.[6,16-18,39,90,91] For the other categories of mobile sources, the estimate of total PAH emission can be based on the heavy-duty spark-ignition or heavy-duty diesel emission rate per gallon of fuel consumed and the total fuel consumption of the category (railroads, aircraft, etc.). When more than one value for a particular PAH emission rate is available for a source category, the micrograms-per-gallon-of-fuel figures are averaged. Kraft and Lies found a very similar distribution of the PAHs for diesel and gasoline vehicles. Owing to the paucity of emission-rate measurements, we used this observation to derive emission factors for vehicle categories when measurements are lacking. Table 1-9 lists the resulting emission-rate estimates for the different vehicle categories. The assumptions made in deriving these estimates were:

- That the PAH distributions for both spark-ignition and diesel vehicles are the same as the distributions for average light-duty spark-ignition noncatalyst vehicles.
- That the measured BaP emission rates for the oxidation-catalyst spark-ignition vehicles and for the three-way-catalyst classes represent the reductions in all PAH emission rates, compared with the noncatalyst values.
- That the heavy-duty spark-ignition class has the same fuel-specific emission rates (in micrograms per gallon) as the light-duty spark-ignition noncatalyst vehicles.
- That the heavy-duty diesel class has the same fuel-specific emission rates as the light-duty diesel class.

Within the limits of those assumptions, we have a complete list of PAH emission factors for each of the vehicle categories in terms of micrograms per gallon of fuel consumed. With typical fuel-economy values for each class, one can calculate the micrograms per mile for each class. These results are also listed in Table 1-9. The 1-nitropyrene values are those from actual experimental measurements, unless a derived value was higher than the measured value. Therefore, the resulting 1-nitropyrene emission rate should be considered an upper limit.

Fuel-specific PAH emission rates can be combined with the total fuel-consumption values in Table 1-3. That yields a total emission tonnage for each mobile-source category for each PAH and PAH derivative in Table 1-9. The PAHs released from mobile sources in 1979 according to these estimates are listed in Table 1-10. The total BaP emission from all mobile sources is estimated to be 43 metric tons. This encompasses all mobile sources, whereas the motor-vehicle contribution is 27 metric tons (about 63% of the total) exclusive of the railroad, aircraft, ship, farm, military, and other contributions. Motor-vehicle BaP emission was estimated in 1972 at about 20 metric tons/yr.[62] The calculated mobile-source emission of 1-nitropyrene is 17 metric tons, of which 30% is calculated to be contributed by motor vehicles. The non-motor-vehicle categories tend to be less relevant to polluted-air concentrations, because they are used away from urban areas (railroads, ships, farm machinery) or because their emission is dispersed above the boundary layer (aircraft). In addition, the various motor-vehicle categories are used to various extents in urban areas. Passenger-car use is 60% urban, light-truck use 55% urban, and heavy-truck use only 20% urban.[60] The urban fraction of the total motor-vehicle PAH emission is calculated to be 63% for 1979, owing mainly to the dominance of noncatalyst-passenger-car emission of PAHs. The relative contributions of each of the mobile-source categories to PAH and 1-nitropyrene emission are listed in Table 1-11. The 70% contribution of the non-motor-vehicle sources to the 1-nitropyrene emission may be an artifact of the method used for calculating emission rates, inasmuch as 1-nitropyrene from sources other than passenger cars and trucks has not been investigated.

By far the largest single contribution to PAH emission (from mobile sources) is that from noncatalyst spark-ignition passenger cars, which will soon be supplanted by vehicles equipped with oxidation catalysts and three-way catalysts. The second most important category for motor-vehicle PAH emission consists of spark-ignition light trucks. As much as half the spark-ignition light trucks (all those under 8,500 lb) will have catalysts by the year 2000. The relative importance of the heavy-truck diesels can be expected to increase with that of the light-truck diesel and passenger-car diesel categories.

Comparison of the estimated BaP emission factors in Table 1-9 for each of the motor-vehicle categories with values reported in the literature indicates that the present estimates tend to be on the high side of what might be expected for fleet-average values. (For the purpose of the estimates in this report, overestimates are obviously preferable to underestimates.) For example, light-duty diesel BaP emission rates range from less than 1 μg/mi to more than 20 μg/mi, with mean values reported in the vicinity of 3-4 μg/mi. The present estimate is 13 μg/mi for light-duty diesels. The few measurements of BaP emission rates for heavy-duty diesels that have been reported[18] indicate that the 54-μg/mi value in Table 1-9 may be too high by as much as an order of magnitude. The reason for this discrepancy is not apparent, but it may reflect a real difference between the four-stroke indirect-injection light-duty diesel and the two- or four-stroke direct-injection heavy-duty diesel. If the lower emission rates are correct, the role of heavy-duty diesel emission is considerably less than portrayed in later sections of this report.

It is now possible to use the emission rates in Table 1-9 and the projections previously described to estimate future rates of emission from motor vehicles. Using the current BaP emission rates, we have calculated the motor-vehicle BaP emission for the year 2000 and listed the results in Table 1-12. The 24 metric tons of BaP represents an 11% decrease from the 1979 value of 27 metric tons and reflects the benefit of catalyst-equipped spark-ignition passenger cars over their noncatalyst counterparts, which is partially offset by the incursion of diesel vehicles. In the year 2000, without further particulate-emission controls, diesel vehicles will account for 40% of the mileage, 50% of the fuel consumption, and 80% of the total motor-vehicle BaP emission, according to this estimate. If the present distribution of motor-vehicle use between urban and rural areas will still be valid in the year 2000, it can be estimated that about 4[illegible] of the BaP from motor vehicles will be released in urban areas in the year 2000, compared with 63% in 1979. Thus, the BaP tonnage nationwide will decrease slightly and there will be a shift to more rural emission and away from urban areas.

The total-tonnage estimates just described do not assess directly the problem of human exposure to air pollutants. In this regard, emission rates are not the sole important quantities. What is needed is an estimate of atmospheric concentrations in the air inhaled by people. The results of atmospheric-dispersion modeling by Ingalls and Garbe can be used to calculate atmospheric concentrations resulting from motor-vehicle emission in many of the typical urban-exposure situations.[45] The model was constructed on the basis of a hypothetical 1-g/mi traffic-weighted emission rate for 1980 vehicle distributions. To calculate atmospheric concentrations for an emission component with other than a 1-g/mi emission rate, one need only multiply the Ingalls and Garbe exposure-concentration factor by the actual traffic-weighted emission rate in grams per mile. Table 1-13 lists the exposure conditions modeled by Ingalls and Garbe and the 1-g/mi exposure-concentration factors derived. One can calculate exposure to BaP on the basis of the data in Tables 1-13 through 1-15. In the calculation, the effect of binding of BaP to particles on deposition and absorption is not considered. Because retention of particle-bound BaP, and thus absorption, depends heavily on particle size and because particle size varies widely, we have characterized exposure on the assumption of complete retention. We assume that 90% of the BaP is bound to particles less than 1 μm in diameter.[72] To use these results for calculation of BaP exposures, one uses Table 1-14 to derive the traffic-weighted BaP emission rate for 1979. The same data for the year 2000 are listed in Table 1-15. The 1979 exposure concentration of BaP in a typical roadway tunnel is 0.017 $\mu g/m^3$ [$(15.3\ \mu g/mi)(10^{-6}\ g/\mu g)(1{,}123\ g/m^3$ per $g/mi)$]. A person exposed to a concentration of 0.017 $\mu g/m^3$ for 2 min while breathing at the rate of 15 m^3/d would inhale 0.4 ng of BaP [$(0.017\ \mu g/m^3)(15\ m^3/d)(1/24)(1/60)(2\ min)(10^3\ ng/\mu g)$]. The total daily exposure of a person can be calculated by summing over each of the exposure situations experienced in the course of the day. The result of these calculations is a degree of exposure by inhalation. The dose of BaP to the body would be less and would depend on the fraction of the BaP-laden particles that is deposited in the body. This fraction is highly uncertain and depends on particle size, shape, and hygroscopicity and on BaP loading per particle, which also depends on particle size. The fraction of BaP deposited is probably about 20-50% of the BaP inhaled. This has been done in Table 1-16 for a person living in a suburb (1,000 m from an expressway) with a 1-h commute to a job at street level in a central-city street canyon. These are rather severe conditions and result in higher exposures than would be expected for the average urban dweller. These conditions lead to a calculated inhalation of 20 ng of BaP. Had the person stayed home all day, the exposure would have resulted in a 3.0-ng inhalation. For comparison, the BaP inhalation from one cigarette is 20 ng.[62] Smoking 1 cigarette/d has the effect of being exposed to over 15 ng/m^3 for the entire day (see discussion on BaP exposure from smoking in Appendix C)--an inhalation of over 330 ng of BaP. For the traffic composition projected to exist in the year 2000, the calculated workday exposure of the person is 9.1 ng of BaP, a 55% decrease from the 1979 value. This shows again the decrease in urban exposure at the expense of an increase in rural exposure. The 9.1-ng BaP inhalation when combined with the 15-m^3/d inhalation rate gives a calculated average daily atmospheric concentration of 0.6 ng/m^3 in the year 2000.

Calculations of BaP total motor-vehicle emission tonnage, urban fractions, and inhalation exposures for the year 2000 have assumed no changes from the present fuel-specific emission rates for the various motor-vehicle categories. Even modest particulate-emission controls (50%

reductions) for diesel cars and trucks and for spark-ignition light trucks would result in significantly greater reductions in BaP (and other PAH) exposures than those calculated here. This of course assumes that compliance (i.e., not removing or poisoning catalytic converters) approaches 100%. The benefit of such controls on the basis of BaP exposures will need to be assessed.

FUTURE CONTROL TECHNOLOGIES

The need for control of emission from light-duty spark-ignition vehicles seems to be moot, with all new vehicles being sold now having catalyst systems. Use of catalysts on heavy-duty spark-ignition vehicles, if feasible, would be expected to result in PAH reductions comparable with those observed for light-duty spark-ignition vehicles. Control of diesel particulate material has received much attention recently. The light-duty diesel particulate-emission standard of 0.6 g/mi that went into effect for the 1982 model year was achieved by most diesel manufacturers through engine modifications. The 0.2-g/mi standard proposed for 1985 would not be as readily attained, at least for the larger vehicles. Currently, only diesels of less than 2,600 lb could meet a 0.2-g/mi particulate-emission standard without exceeding the gaseous-emission standards.[85] Variations in diesel fuel appear to be inadequate to allow attainment of the standard for all but the smaller diesel vehicles.

A number of diesel-particle control techniques are under investigation. In general, these entail after-treatment devices designed to collect particles from the exhaust stream and to oxidize the collected material periodically. Diesel-particle control devices are being developed by several companies and are being tested by automobile manufacturers. Texaco has reported results on an alumina-coated metal-wool diesel-particle filter that achieved a collection efficiency of about 70% without increasing the backpressure enough to sacrifice fuel economy and performance. The collected particulate material must then be removed by combustion.[57,69,87] Johnson-Matthey is developing a wire-mesh particle trap that is coated with a catalytic material to initiate the combustion of the collected soot. Efficiencies exceeding 50% have been achieved during 50,000-mi accumulation with regeneration every 300-1,000 mi. Corning has developed a ceramic-honeycomb monolithic particle filter that can be coated with a catalyst material to assist in soot combustion.[87] Gorse et al.[29] and Williams[89] recently reported emission characterization studies that used some of the above-mentioned control devices. The ceramic trap removes more than 90% of the elemental carbon particles and about 50% (with the catalyst coating) of the particulate organic material and can result in an order-of-magnitude decrease in the emission of bacterial mutagens per mile of travel The wire-mesh catalyst trap removes more than 90% of the particulate organic material and 30% of the elemental carbon. Some of the catalyst-coated traps can produce very high sulfate emission rates, especially during regeneration.

In general, there appears to be some hope of success for diesel-particle control, but the devices tested so far need to be tested for durability, packaging, and on-road reliability. None of the devices has been evaluated for use with heavy-duty diesel particle emission. If diesel-particle control devices are successfully developed and used on light- and heavy-duty diesel vehicles, reductions in the PAH emission factor by at least a factor of 2, and conceivably a factor of 10 or better, could be realized.

Cost estimates of the diesel-particle control techniques are premature; the technology of choice has not yet been determined. Johnson-Matthey claims that the cost of its device could be as low as $150 for light-duty diesels.[36] General Motors estimates that the cost of light-duty diesel-particle control could be as high as $830/vehicle to meet the 0.2-g/mi particle standard and the 1.0-g/mi NO_x standard at low and high altitude.[23] The control device itself would represent about half the total cost, and the control modifications needed to ensure the functioning of the trap would represent the other half.

TABLE 1-1

U.S. Energy Consumption, 1979[a]

Energy Source	Energy Content, 10^4 BTU/unit	Consumption	
		10^{15} BTU	Quantity
Oil	9.8/gal	34.2	3.5×10^{11} gal
Coal	1.3/lb	15.6	1.2×10^{12} lb
Natural gas	0.1/ft^3	20.4	2.0×10^{13} ft^3
Hydrogeneration	--	3.1	--
Nuclear power	--	2.7	--
Wood	1.0/lb	0.17	1.7×10^{10} lb
Other	--	0.12	--
Total		76	

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[a] Data from Motor Vehicle Manufacturers Association.[60]

TABLE 1-2

Uses of Major Crude-Oil Fractions in the United States, 1979[a]

Destination	Consumption, 10^9 gal Gasoline	Distillate Oil	Residual Oil	Other[b]
Residential	--	17	--	0.8
Commercial	--	5.9	5.1[c]	0.1
Industrial	--	6.2	11	0.1
Electric utility	--	2.1	22	--
Oil company	--	1.1	3.3	--
Farm	1.3	4.3	--	--
Military	--	0.9	0.2	--
Rail	--	4.9	0.01	--
Marine	--	2.1	7.7	--
Highway vehicles	104.2	8.6[d]	--	--
Off-highway vehicles	1.6	3.4	--	--
Other	5.5	1.8	1.0	100.0
Totals[e]	112.6	58.3	50.3	101.0
[f]	141.9	66.4	56.7	106.5

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[a] Data from Motor Vehicle Manufacturers Association[60] and *National Petroleum News*.[61]

[b] Includes jet fuels, kerosene, lubricants, asphalt, etc.

[c] Combined total for residential and commercial categories.

[d] Federal Highway Administration data show 18.3 x 10^9 gal for highway use, which is compatible with Table 1-3.

[e] Compiled from Department of Energy data.

[f] Data from University of Houston Downtown College, Energy Information Services, *U.S. Annual Energy Facts*.

TABLE 1-3

Mileage and Fuel Consumption, 1979[a]

Fuel User	Fuel	Mileage, 10^{10} mi/yr	Fuel Consumption, 10^9 gal/yr
Highway vehicles:			
Passenger cars	Gasoline	112.9	79.0
	Diesel	1.1	0.81
Trucks	Gasoline	28.8	23.2
<33,000 lb	Diesel	0.59	0.74
Trucks	Gasoline	0.34	1.1
>33,000 lb	Diesel	6.4	15.9
Buses	Gasoline	0.30	0.41
	Diesel	0.31	0.62
Motorcycles	Gasoline	2.2	0.44
Other:			
Railroads	Diesel	--	4.4
Ships	Gasoline	--	0.93
	Diesel	--	8.7
Aircraft	Gasoline	--	0.77
	Jet	--	22.0
Farm vehicles	Gasoline	--	1.3
	Diesel	--	4.3
Military vehicles	Diesel	--	0.85
Other	Gasoline	--	5.4
	Diesel	--	3.5
Totals	Gasoline	152.9	112.6
	Diesel	--	39.8
	Jet	--	22.0

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[a] Data from Jambekar and Johnson,[46] Motor Vehicle Manufacturers Association,[60] National Petroleum News,[61] and Shelton.[82,83]

TABLE 1-4

Exhaust Emission Rates for Light-Duty Gasoline-Powered Vehicles[a]

Emission Component	Model Year	Zero-Mile Emission Rate, g/mi	50,000-Mile Emission Rate,[b] g/mi
HC	Pre-1968	7.25	8.15
	1968-1969	4.43	5.68
	1970-1971	3.00	4.85
	1972-1974	3.36	4.21
	1975-1979	1.29	2.74
	1980	0.29	1.74
	1981	0.39	1.34
	1982+	0.39	1.34
CO	Pre-1968	78.27	89.52
	1968-1969	56.34	69.09
	1970-1971	42.17	57.82
	1972-1974	40.78	52.98
	1975-1979	20.16	34.46
	1980	6.14	20.44
	1981	5.60	19.35
	1982	5.21	19.01
	1983+	5.00	18.80
NO_x	Pre-1968	3.44	3.44
	1968-1972	4.35	4.35
	1973-1974	2.87	3.07
	1975-1976	2.43	2.63
	1977-1979	1.69	2.19
	1980	1.56	2.06
	1981+	0.75	1.50

---

[a] Data from U.S. Environmental Protection Agency.[84] Emission rates are for low-altitude 49-state vehicles. High-altitude and California emission rates are different.

[b] The 50,000-mile emission rates are calculated from zero-mile rate by addition of term that takes account of EPA-projected deterioration rate of vehicle combustion and emission-control systems.

TABLE 1-5

Summary of Gaseous Hydrocarbons Emitted from Vehicles

All n-alkanes from n-butane through n-hexacosane
Four methyl-substituted butanes
Ten methyl- and ethyl-substituted pentanes and 11 cyclopentanes
Eleven methyl- and ethyl-substituted hexanes and 35 cyclohexanes
Fifteen methyl- and ethyl-substituted heptanes
Five methyl-substituted octanes
One methyl-substituted nonane
One methyl-substituted decane
One methyl-substituted undecane
Decalin and two methyl-substituted decalins
Two C_{10} alkanes
Eleven C_{11} alkanes
Nine C_{12} alkanes
Thirteen C_{13} alkanes
Eleven C_{14} alkanes
Eight C_{15} alkanes
Eight C_{16} alkanes
Five C_{17} alkanes
Three C_{18} alkanes
Seven methyl-substituted butenes and two methyl butadienes
Eighteen pentenes and pentadiene
Fourteen hexenes
Six heptenes
Four octenes
Decene and dodecene through heneicosene
Seven cyclic olefins
Seventy-one alkyl-substituted benzenes
Eight styrenes and the three xylenes
Fourteen indans and three indenes
Twenty-eight alkyl-substituted naphthalenes
Three alkylthiophenes and two benzothiophenes
Two alkylsulfides and one alkylamine
Six nonaromatic alcohols and eight aromatic alcohols
Eighteen aliphatic and aromatic aldehydes
Six furans, 17 ketones, and six organic acids

TABLE 1-6

Qualitative Analysis of Nonpolar and Moderately Polar Fractions of Diesel Particulate Extract

Compounds	Approximate Concentration in Oldsmobile Extract, ppm	
Nonpolar fractions:		
Phenanthrenes and anthracenes	600	
Methylphenanthrenes and methylanthracenes	1,400	
Dimethylphenanthrenes and dimethylanthracenes	3,000	
Pyrene	1,700	
Fluoranthene	1,400	
Methylpyrenes and methylfluoranthenes	800	
Chrysene	100	
Cyclopenta[cd]pyrene	20	
Benzo[ghi]fluoranthene	100	
Benz[a]anthracene	500	
Benzo[a]pyrene	40	
Other PAHs, heterocyclics	30,000	
Hydrocarbons and alkylbenzenes	500,000	
Total, nonpolar fractions		539,700
Moderately polar fractions:		
PAH ketones:		
Fluorenones	4,000	
Methylfluorenones	400	
Dimethylfluorenones	200	
Anthrones and phenanthrones	1,600	
Methylanthrones and methylphenanthrones	1,600	
Dimethylanthrones and dimethylphenanthrones	1,300	
Fluoranthones and pyrones	1,200	
Benzanthrones	200	
Xanthones	300	
Methylxanthones	200	
Thioxanthones	1,600	
Methylthioxanthones	900	
		13,500

TABLE 1-6 (continued)

Compound	Approximate Concentration in Oldsmobile Extract, ppm	
PAH carboxaldehydes:		
Fluorene carboxaldehydes	1,600	
Methyl fluorene carboxaldehydes	400	
Phenanthrene and anthracene carboxaldehydes	2,600	
Methylanthracene and methylphenanthrene carboxaldehydes	1,600	
Dimethylanthracene and dimethylphenanthrene carboxaldehydes	400	
BaA, chrysene, and triphenylene carboxaldehydes	400	
Naphthalene dicarboxaldehydes	300	
Dimethylnaphthalene carboxaldehydes	300	
Trimethylnaphthalene carboxaldehydes	1,000	
Pyrene and fluoranthene carboxaldehydes	1,600	
Xanthene carboxaldehydes	600	
Dibenzofuran carboxaldehydes	400	
		11,200
PAH acid anhydrides:		
Naphthalene dicarboxylic acid anhydrides	3,000	
Methylnaphthalene dicarboxylic acid anhydrides	1,000	
Dimethylnaphthalene dicarboxylic acid anhydrides	500	
Anthracene and phenanthrene dicarboxylic acid anhydrides	600	
		5,100
Hydroxy PAHs:		
Hydroxyfluorene	1,400	
Methylhydroxyfluorene	400	
Dimethylhydroxyfluorene	1,500	
Hydroxyanthracenes and hydroxyphenanthrenes	600	
Hydroxymethylanthracenes and hydroxymethylphenanthrenes	900	
Hydroxydimethylanthracenes and hydroxydimethylphenanthrenes	1,300	
Hydroxyfluorenone	2,000	
Hydroxyxanthone	1,300	
Hydroxyxanthene	1,000	
		10,400

TABLE 1-6 (continued)

Compound	Approximate Concentration in Oldsmobile Extract, ppm	
PAH quinones:		
Fluorene quinones	700	
Methylfluorene quinones	600	
Dimethylfluorene quinones	500	
Anthracene and phenanthrene quinones	1,900	
Methylanthracene and methylphenanthrene quinones	2,000	
Fluoranthene and pyrene quinones	200	
Naphtho[1,8-cd]pyrene 1,3-dione	600	
		6,500
Nitro PAHs:		
Nitrofluorenes	30	
Nitroanthracenes and nitrophenanthrenes	70	
Nitrofluoranthenes	10	
Nitropyrenes	150	
Methylnitropyrenes and methylnitrofluoranthenes	20	
		300
Other oxygenated PAHs:	8,000	8,000
PAH carryover from nonpolar fraction:	6,000	6,000
Phthalates, HC contaminants:	30,000	30,000
Total, moderately polar fractions		91,000

TABLE 1-7

Nitroarenes Indicated in Diesel-Exhaust Particulate Extracts

Mononitroarenes:

Nitroindene
Nitroacenaphthylene
Nitroacenaphthene
Nitrobiphenyl
Nitrofluorene
Nitromethylacenaphthylene
Nitromethylacenaphthene
Nitromethylbiphenyl
Nitroanthracene
Nitrophenanthrene
Nitromethylfluorene
Nitromethylanthracene
Nitromethylphenanthrene
Nitrotrimethylnaphthalene
Nitrofluoranthene
Nitropyrene
Nitro(C_2-alkyl)anthracene
Nitro(C_2-alkyl)phenanthrene
Nitrobenzofluorene
Nitromethylfluoranthene
Nitromethylpyrene
Nitro(C_3-alkyl)anthracene
Nitro(C_3-alkyl)phenanthrene
Nitrochrysene
Nitrobenzoanthracene
Nitronaphthacene
Nitrotriphenylene
Nitromethylnaphthacene or
  Nitromethylchrysene
Nitromethylbenzanthracene
Nitromethyltriphenylene
Nitrobenzopyrene
Nitroperylene
Nitrobenzofluoranthene

Polynitroarenes:

Dinitromethylnaphthalene
Dinitrofluorene
Dinitromethylbiphenyl
Dinitrophenanthrene
Dinitropyrene
Trinitropyrene
Trinitro(C_5-alkyl)fluorene
Dinitro(C_6-alkyl)fluorene
Dinitro(C_4-alkyl)pyrene

Nitro-oxyarenes:

Nitronaphthaquinone
Nitrodihydroxynaphthalene
Nitronaphthalic acid
Nitrofluorenone
Nitroanthrone
Nitrophenanthrone
Nitroanthraquinone
Nitrohydroxymethylfluorene
Nitrofluoranthone
Nitropyrone
Nitrofluoranthenequinone
Nitropyrenequinone
Nitrodimethylanthracene
  carboxaldehyde
Nitrodimethylphenanthrene
  carboxaldehyde

Other nitrogen compounds:

Benzocinnoline
Methylbenzocinnoline
Phenylnaphthylamine
(C_2-Alkyl)phenylnaphthylamine

TABLE 1-8

Measured PAH Emission Rates for Mobile Sources

	Emission Rate, µg/gal of fuel consumed												
	Spark Ignition												
	Light Duty							Heavy Duty	Diesel, Noncatalyst				
	Leaded Fuel					Unleaded Fuel		Leaded Fuel					
	Noncatalyst					Oxidation Catalyst	Three-Way Catalyst	Noncatalyst	Light Duty				Heavy Duty
PAH	a	b	c	d	Avg.	a	a	e	f	g	i	Avg.	g
Anthracene	2,251	2,226			2,239								
Phenanthrene	8,163	10,005			9,084								
Methylphenanthrene		5,678			5,678								
Dimethylfluorene		2,972			2,972								
Dimethylphenanthrene		2,014			2,014								
Fluoranthene	4,221	5,152		4,900	4,758	96	12		2,311		4,400	4,400	
Pyrene	5,066	9,528	331	3,500	6,031				13,636		4,320	6,689	
Benzofluorene		750			750								
Benzoanthracene	231				231								
Triphenylene		189			189								
Cyclopentapyrene	1,653	3,290			2,472						1,160	1,160	
Chrysene	1,689	394		735	939								
Indenofluoranthene		83			83						152	152	
Indenopyrene	281	223		70	191								
Methylchrysene		38			38								
1-Nitropyrene			3.5		3.5	4.3	0.4		212 379	222		271	48
Benzofluoranthene	422	920		665	669						840	840	
Benzo[e]pyrene	338	182		455	325						360	360	
Benzo[a]pyrene	281	250	261	298	273	50	5.9	198	15 832	135	300	321	133
Perylene		42		11	27						16	16	
Cyclopentabenzopyrene		163			163								
Benzochrysene		4			4								
Anthracene	197	83			140								
Dibenzanthracene	79				79								
Benzoperylene	1,126	868		196	723						340	340	
Coronene	704	715		88	502						84	84	
Cyclopentabenzoperylene		114			114								

Footnotes to Table 1-8:

[a] Data from Grimmer.[33]

[b] Data from Grimmer *et al*.[34]

[c] Data from Lang *et al*.[51]

[d] Data from Kraft and Lies.[48]

[e] Data from Dietzmann *et al*.[16-18]

[f] Data from Braddock.[6]

[g] Data from Williams and Chock,[90] Hare and Baines,[39] and Williams and Swarin.[91]

TABLE 1-9

Derived PAH Emission Rates for Mobile Sources

PAH	Emission Rate[a]													
	Spark Ignition													
	Light Duty						Heavy Duty							
	Leaded Fuel		Unleaded Fuel				Leaded Fuel		Diesel, Noncatalyst				Motorcycles, Leaded Fuel	
	Noncatalyst		Oxidation Catalyst		Three-Way Catalyst		Noncatalyst		Light-Duty		Heavy Duty			
	µg/gal	µg/mi	µg/gal	µg/mi	µg/gal	µg/mi	µg/gal	µg/mi	µg/gal	µg/mi	µg/gal	µg/mi	µg/gal	µg/mi
Anthracene	2,239	160	410	23	49	3	2,239	448	2,633	105	2,633	439	2,239	45
Phenanthrene	9,084	649	1,664	92	196	10	9,084	1,817	10,681	427	10,681	1,780	9,084	182
Methylphenanthrene	5,678	406	1,060	58	123	6	5,678	1,136	6,676	267	6,676	1,113	1,113	116
Dimethylfluorene	2,972	212	544	30	64	3	2,972	594	3,495	140	3,495	583	2,972	59
Dimethylphenanthrene	2,014	144	369	21	46	2	2,014	403	2,368	95	2,368	395	2,014	40
Fluoranthene	4,758	340	871	48	103	5	4,758	952	5,595	224	5,595	933	4,758	95
Pyrene	6,031	431	1,105	61	130	7	6,031	1,206	7,091	284	7,091	1,182	6,031	121
Benzofluorene	750	54	137	8	16	0.8	750	150	882	35	882	147	750	15
Benzanthracene	231	17	42	2	5	0.3	231	46	272	11	272	45	231	5
Triphenylene	189	14	35	2	4	0.2	189	38	222	9	222	37	189	4
Cyclopentapyrene	2,472	177	453	25	53	3	2,472	494	2,907	116	2,907	485	2,472	49
Chrysene	939	67	172	10	20	1	939	188	1,106	44	1,106	184	939	19
Indenofluoranthene	83	6	15	0.8	2	0.1	83	17	98	4	98	16	83	2
Indenopyrene	191	14	35	2	4	0.2	191	38	225	9	225	38	191	4
Methylchrysene	38	3	7	1.4	1	0.1	38	8	45	2	45	8	38	0.8
1-Nitropyrene	3.5	0.3	4	0.2	0.4	0.0	4	0.8	271	11	271	45	3.5	0.1
Benzofluoranthene	669	48	123	7	15	0.8	669	134	840	34	840	140	669	14
Benzo[e]pyrene	325	23	60	3	7	0.4	325	65	382	15	382	64	325	7
Benzo[a]pyrene	273	20	50	3	6	0.3	273	55	321	13	321	54	273	5
Perylene	27	2	5	0.3	1	0.0	27	5	32	1	32	5	27	0.5
Cyclopentabenzopyrene	163	12	30	2	4	0.2	163	33	192	8	192	32	163	3
Benzochrysene	4	0.3	1	0.1	0.1	0.0	4	0.8	5	0.2	5	0.8	4	0.1
Anthanthrene	140	10	26	1	3	0.2	140	28	165	7	165	28	140	3
Dibenzanthracene	79	6	14	0.8	2	0.1	79	16	93	4	93	16	79	2
Benzoperylene	723	52	132	7	16	0.8	723	145	850	34	850	142	723	14
Coronene	502	36	92	5	11	0.6	502	100	590	24	590	98	502	10
Cyclopentabenzo-perylene	114	8	21	1	2	0.1	114	23	136	5	136	22	114	2

---

[a] µg/gal = µg of PAH per gallon of fuel consumed. µg/mi = (µg/gal)/(mi/gal).

TABLE 1-10

Estimated PAH Emission from Mobile Sources, 1979

PAH	Total Emission, metric tons
Anthracene	350
Phenanthrene	1,400
Methylphenanthrene	900
Dimethylfluorene	470
Dimethylphenanthrene	320
Fluoranthene	750
Pyrene	950
Benzofluorene	120
Benzanthracene	37
Triphenylene	30
Cyclopentapyrene	390
Chrysene	150
Indenofluoranthene	19
Indenopyrene	30
Methylchrysene	6
1-Nitropyrene	17
Benzofluoranthene	110
Benzo[e]pyrene	52
Benzo[a]pyrene	43
Perylene	4
Cyclopentabenzopyrene	26
Benzochrysene	1
Anthanthrene	22
Dibenzanthracene	13
Benzoperylene	110
Coronene	80
Cyclopentabenzoperylene	18
Total[a]	6,400

---

[a] Total for all PAHs: about (3)(6,400) = about 19,000 metric tons. Benzo[a]pyrene from mobile sources is therefore about 0.2% of total PAHs.

TABLE 1-11

Contributions of Mobile-Source Categories to PAH and 1-Nitropyrene Emission, 1979

Category	PAH Emission, %		1-Nitropyrene Emission, %	
Motor vehicles:				
Passenger cars:				
Noncatalyst	29.1		0.9	
Oxidation catalyst	3.8		0.6	
Diesel	0.6		1.3	
Trucks, <33,000 lb:				
Spark-ignition	14.7		0.5	
Diesel	0.6		1.2	
Trucks, >33,000 lb:				
Spark-ignition	0.7		0.02	
Diesel	11.8		25.1	
Buses:				
Spark-ignition	0.3		0.01	
Diesel	0.6		1.0	
Motorcycles	0.3	62.5	0.01	30.6
Other mobile sources:				
Railroads	3.3		6.9	
Ships	7.1		13.7	
Aircraft	16.8		34.7	
Farm	4.0		6.8	
Military	0.6		1.3	
Miscellaneous	6.0	37.8	5.6	69.0
		100.3		99.6

TABLE 1-12

Projected Vehicle Use and Benzo[a]pyrene Emission for the Year 2000

Vehicle Type	Fuel	Mileage, 10^{10} mi/yr	Fuel Economy, mi/gal	Fuel Consumption, 10^9 gal/yr	Benzo[a]pyrene Emitted, metric tons
Passenger Cars	Gasoline	101.2[a]	30	33.7	1.0
	Diesel	39.0	40	9.8	3.1
Trucks, <33,000 lb	Gasoline	50.0[b]	20	25.0	3.9
	Diesel	50.0	30	16.7	5.4
Trucks, >33,000 lb	Diesel	18.8	6	31.3	10.0
Buses	Gasoline	0.4	7	0.6	0.2
	Diesel	1.7	10	1.7	0.5
Motorcycles	Gasoline	3.0	50	0.6	0.2
Totals		264		119	24.3

---

[a] 50% with oxidation catalysts, 50% with three-way catalysts.

[b] 50% with oxidation catalysts, 50% without catalysts.

Assumptions: 1.5%/yr increase in passenger-car and motorcycle mileage.
6%/yr increase in light-truck and bus mileage.
5%/yr increase in heavy-truck mileage.
Fuel-economy improvements, as listed in column 4.
Benzo[a]pyrene emission rates (μg/gal) unchanged from values in Table 1-9.

TABLE 1-13

Summary of Microscale Exposure-Concentration Factors[a]

Exposure Situation	Exposure-Concentration Factor,[b] g/m^3 per 1 g/mi	
1. Residential garage:		
Typical (30-s run time)	7,900	
Severe (5-min run time)	67,000	
2. Parking garage:		
Typical (parking level)	3,900	
Severe: inlet-air component	9,600	
exhaust-emission component	46,100	
3. Roadway tunnel:		
Typical	1,123	
Severe	2,856	
4. Street canyon (sidewalk receptor, includes background):		
Typical: 800 vehicles/h	42	
1,600 vehicles/h	85	
Severe: 1,200 vehicles/h	141	
2,400 vehicles/h	282	
5. On expressway (wind: 315 deg relative, 2.2 mph):		
Typical	124	
Severe	506	
6. Beside expressway:	Short-Term	Annual
Severe: 1 m	397	61
10 m	334	48
100 m	105	14
1,000 m	13.6	1.6

---

[a] Adapted from Ingalls and Garbe.[45]

[b] For 1 g/vehicle-mile (1 g/vehicle-minute for idle conditions). Assumes no background concentrations except as noted. To use these values with emission factors other than 1 g/mi (or 1 g/min), multiply the concentration factor by the actual emission factor in grams per mile (or grams per minute for idle conditions).

TABLE 1-14

Urban Motor-Vehicle Data, 1979

Vehicle Type	Urban Mileage Fraction	Fuel Economy, mi/gal	Fuel Consumption, 10^9 gal	Benzo[a]pyrene, metric tons
Passenger cars:				
Noncatalyst, spark-ignition	0.6	13.5	26.8	7.3
Oxidation catalyst, spark-ignition	0.6	15.4	20.4	1.0
Diesel	0.6	16.3	0.4	0.1
Trucks, <33,000 lb:				
Spark-ignition	0.55	12.4	12.7	3.5
Diesel	0.55	8.0	0.4	0.1
Trucks, >33,000 lb:				
Spark-ignition	0.2	3.1	0.3	0.08
Diesel	0.2	4.0	3.3	1.1
Buses:				
Spark-ignition	0.6	7.3	0.3	0.08
Diesel	0.6	5.0	0.4	0.1
Motorcycles	0.8	50.0	0.4	0.1
Totals			65.4	13.46

Urban-traffic weighted benzo[a]pyrene emission rate:

$$\frac{13.46 \text{ metric tons}}{88 \times 10^{10} \text{ total urban miles}} = 15.3\ \mu g/mi.$$

TABLE 1-15

Urban Motor Vehicle Data, 2000

Vehicle Type	Urban Mileage Fraction	Fuel Economy, mi/gal	Fuel Consumption, 10^9 gal	Benzo[a]pyrene, metric tons
Passenger cars:				
Noncatalyst, spark-ignition	0.6	30	10.1	0.5
Oxidation catalyst, spark-ignition	0.6	30	10.1	0.06
Diesel	0.6	40	5.9	1.9
Trucks, <33,000 lb:				
Noncatalyst, spark-ignition	0.55	20	6.9	0.3
Oxidation catalyst, spark-ignition	0.55	20	6.9	1.9
Diesel	0.55	30	9.2	3.0
Trucks, >33,000 lb:				
Spark-ignition	0	—	0	—
Diesel	0.2	6	6.3	2.0
Buses:				
Spark-ignition	0.6	7	0.3	0.08
Diesel	0.6	10	1.0	0.3
Motorcycles	0.8	50	0.5	0.1
Totals			57.2	10.14

Urban-traffic weighted benzo[a]pyrene emission rate:

$$\frac{10.14 \text{ metric tons}}{146.7 \times 10^{10} \text{ total urban miles}} = 6.9\ \mu\text{g/mi.}$$

TABLE 1-16

Calculation of Benzo[a]pyrene Inhalation Exposure for 1979

Exposure Situation	Exposure Time	Benzo[a]pyrene Concentration, ng/m^3	Benzo[a]pyrene Inhalation,[a] ng
Typical roadway tunnel	2 min	17.2	0.4
Severe roadway tunnel	2 min	43.7	0.9
Typical street canyon, 1,600 veh/h	8 h	1.3	6.5
Severe street canyon, 2,400 veh/h	20 min	4.3	0.9
Severe expressway	2 h	7.7	9.6
Beside expressway	14 h	0.2	1.8
Total exposure			20.1[b]

---

[a] Based on inhalation rate of 15 m^3/d.

[b] Corresponds to daily average atmospheric BaP concentration of 1.3 ng/m^3 [(20.1 ng/d)/(15 m^3/d)].

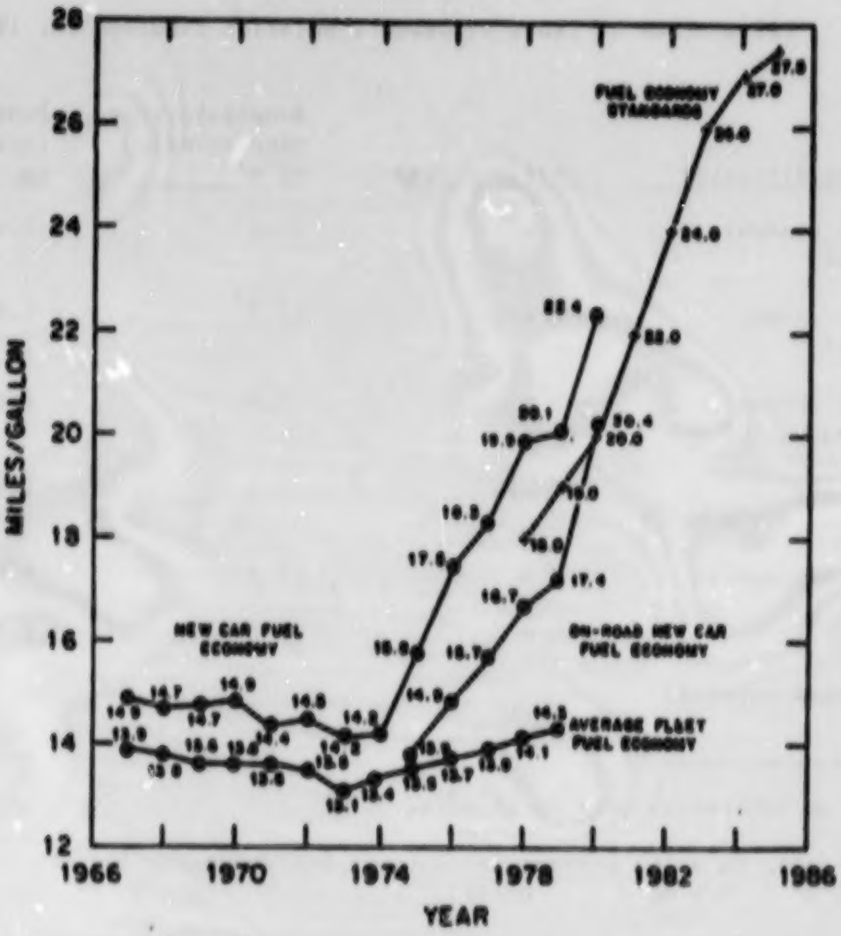

FIGURE 1-1. Automotive fuel economy standards, 1967-1985. Adapted from Kulp *et al.*[49] Based on manufacturers' sales projections (on-road fuel economy based on actual sales).

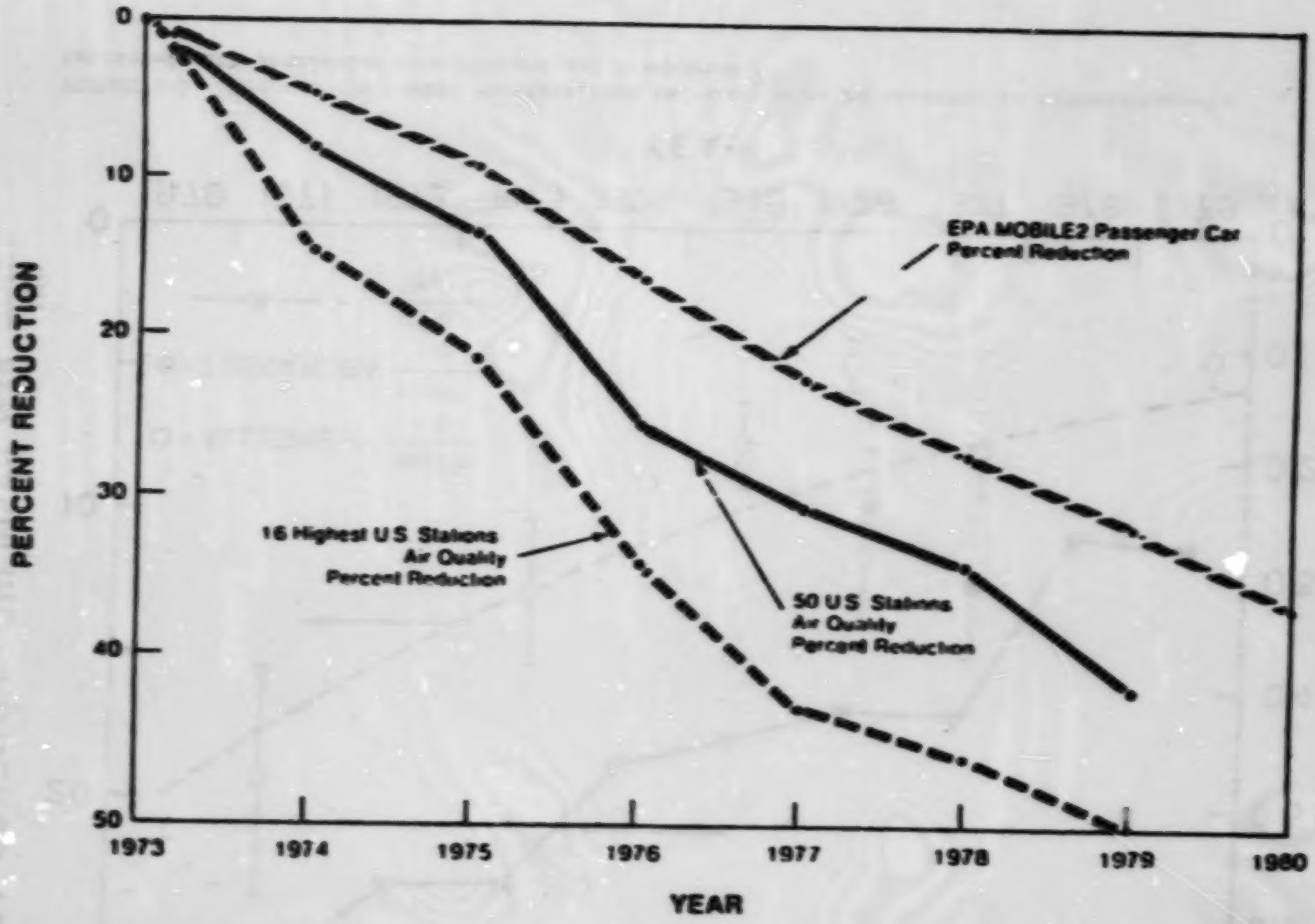

FIGURE 1-2. CO air-quality and emission-factor trend. Base year, 1973. Air-quality reductions are reductions in highest 8-h CO yearly concentration averaged over 50 and 16 important U.S. locations. Adapted from Chang et al.[9]

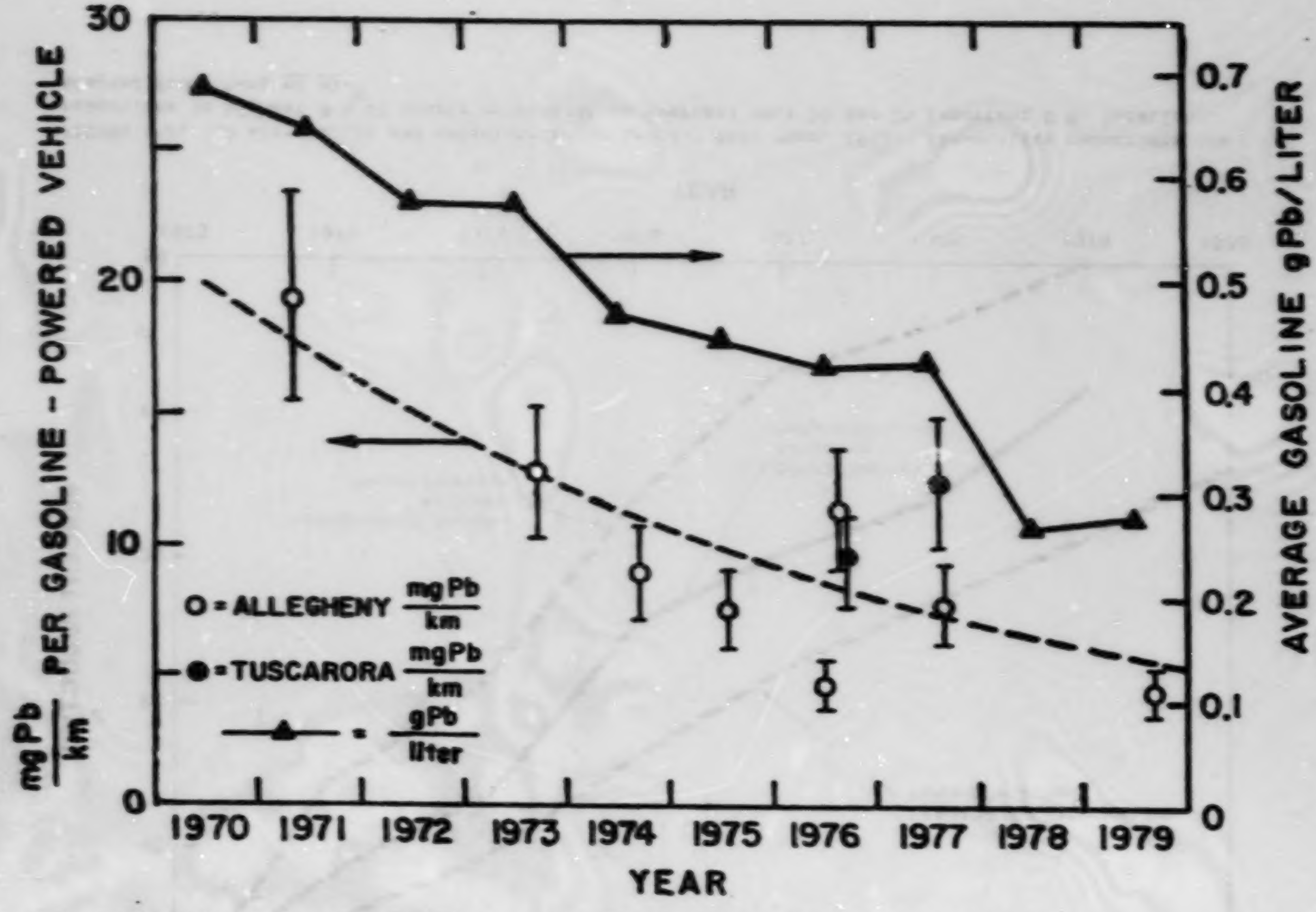

FIGURE 1-3. Decrease in traffic-average lead emission rates as measured in highway tunnels. Reprinted with permission from Pierson and Brachaczek.[72]

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POLYCYCLIC AROMATIC HYDROCARBONS FROM NATURAL AND STATIONARY ANTHROPOGENIC SOURCES AND THEIR ATMOSPHERIC CONCENTRATIONS

Although the emphasis of this report is on the identification of the polycyclic aromatic hydrocarbons (PAHs) emitted from motor vehicles, PAHs are ubiquitous substances. They are found in terrestrial and aquatic plants, in soils and bottom sediments, in fresh and marine waters, in emission from volcanoes and naturally occurring forest fires, and in the products of numerous human activities. The anthropogenic sources vary widely--major oil spills and the inestimable minor spills of petroleum products, emission from coal- and gas-fired boilers and electric-power generating plants, space heaters (especially in individual residences), municipal and industrial incinerators, and all sorts of industrial processes. It is not possible to list all the sources or to count or measure the PAHs produced by them. The various PAH compounds and the amounts emitted into the environment from each of the sources result in a complexity that makes it difficult to trace and identify the major contributing sources.

PAH COMPOUNDS IN PETROLEUM AND FOSSIL-FUEL PRODUCTS

The carcinogenic potential of petroleum hydrocarbons was examined in a critical review of world literature in the period 1960-1978 by Bingham _et al._[3] Although the carcinogenic potential of some samples of petroleum and other fossil-fuel material tested in experimental animals could be associated with the presence of benzo[a]pyrene (BaP), others without benzo[a]pyrene were also carcinogenic. Thus, the authors suggested that benzo[a]pyrene may not be the most prevalent or important component in the samples and recommended further chemical analyses of a variety of petroleum samples to determine the profile of PAH compounds in them. The review included references to the carcinogenicity of high-boiling-point (above 260°C) petroleum fractions, residues, and products and to the occurrence of cancer in workers in refineries and industries in which these materials are used. The review did not discuss the carcinogenicity of pure PAHs or studies of environmental pollution from general sources, as are covered in this report.

PAH COMPOUNDS IN CRUDE OILS, COAL, AND OIL-SHALE DERIVATIVES

Mutagenicity testing (with standard _S. typhimurium_ procedures) by Guerin _et al._[13] of several crude oils and shale- and coal-derived petroleum substitutes showed the petroleum-substitute mutagenicities to be equal to or 10-100 times greater than those of petroleum products.

The nonpolar neutral constituents generally were found to contribute over half the mutagenicity. Those findings give added incentive for identifing and characterizing the individual PAHs in these products.

Crude Oil

In their review of the carcinogenicity of petroleum hydrocarbons, Bingham _et al._[3] cited several of the early researchers' work (1931) on the carcinogenicity of crude-oil fractions from various sources when applied to the skin of mice. The distillate fractions in the 300-400°C range were later found to be more carcinogenic than whole crude oil in mice and rabbits. Although the review cited studies by Hueper in 1965 that failed to produce cancer within the normal life span when undiluted crude oil was applied to C57BL mice, it cited Barr-Nea and Wolman in 1972, who found papillomas after 5-7 mo of topical application of acetone-extracted crude oil. Numerous other studies cited in the review described the carcinogenic potential of crude oils from various locations when tested in animals.

The review by Bingham _et al._ cited references that gave the content of BaP: 40, 1,320, and 1,660 μg/L, respectively, in Persian Gulf, Libyan, and Venezuelan petroleum (Graf and Winter, 1968) and 1,000 and 2,800 μg/kg in South Louisiana and Kuwait crude oils, respectively (Panceron and Brown, 1975).

Several studies cited by McKay and Latham[22] reported qualitative findings of anthracenes, phenanthrenes, benzophenanthrenes, fluorenes, chrysenes, pyrenes, perylenes, and coronene in virgin petroleum. In the process of cracking of petroleum distillates, the high-temperature hydroconversions formed ring systems, such as benzocoronenes, dibenzocoronenes, and tribenzocoronenes. The authors identified seven polynuclear aromatic compounds not previously found in virgin petroleum distillates (temperature, 335-550°C): 1.12,2.3- dibenzoperylene, 1,12-_o_-phenyleneperylene, pyreno[1.3:10'.2']pyrene, 2.3,10.11-dibenzoperylene, 1,2,4,5-dibenzopyrene, benzo[e]pyrene (BeP), and benzo[g]chrysene. Similar quantitative evidence of the presence of pyrene, BaP, BeP, chrysene, and 1,23-benzoperylene was reported by Coleman _et al._[7] in fluorescence emission and fluorescence excitation analysis of Prudhoe Bay crude oil.

Coal and Oil-Shale Derivatives

Coal gasification has been used to produce clean fuels in many countries since 1880. The measurement of individual PAHs in the synthetic oils produced by coal liquefaction and in natural crude oil remains a difficult problem. The samples often are from small-scale processes with questionable resemblance to the products of eventual commercial-scale operations. Guerin _et al._[12] analysed fractions of two coal-derived crude oils (synthoil from catalytic hydrogenation of coal, synthoil C, and syncrude from pyrolysis of coal, syncrude D), shale-derived crude oil shale B, and a petroleum mix (crude-oil mixture from California, Canada, Alaska, Iran, Louisiana-Mississippi, and Arabian Light). The results of the chromatographic analyses are shown in Table 2-1. The coal-derived crudes had larger quantities and a greater variety of PAHs than the petroleum sample, and shale B had less than either of them. The summation of the PAHs produced the following totals: synthoil C, 135 mg/g; syncrude D, 132 mg/g; shale B, 36 mg/g; and petroleum mix A, 58 mg/g.

The increased interest in greater use of petroleum substitutes from oil shale and coal has raised concern about the health hazards associated with these fuels. This concern led Buchanan et al.[5] to study the mutagenicity of subfractions from several fossil fuels, principally the primary aromatic amines (PAAs). Aminofluorene and aminoanthracene were among the PAAs identifed in the subfractions, and azabenzofluorene, azabenzopyrene, and azaanthanthrene were identified in the azaarene group. They mentioned that the mutagenic activity of PAAs is greater than that of azaarenes. Katz and Ogan[17] identified chromatographic peaks of benz[a]anthracene, dibenz[ah]-anthracene, and benzo[ghi]perylene in coal liquid (EPA Chemical Repository Samples CRM-1-3). Earlier, White et al.,[49] during the process of development of gas-chromatographic analysis, used a coal-liquefaction sample from the Synthoil Process Development Unit, Bruceton, Pennsylvania, and identified fluorene, 9,10-dihydrophenanthrene, 1,2,3,4,5,6,7,8-octahydrophenanthrene, 1,2,3,4-tetrahydrophenanthrene, phenanthrene, anthracene, fluoranthene, and pyrene.

Nichols et al.[34] studied raw gases from fixed-bed reactors fueled by different coal or vegetative fuels and collected in a stainless-steel cooler-condenser during the run. The gas-chromatographic analyses of the condensate are reported in Table 2-2 as micrograms per gram of solid feed to the gasifier unit.

USED ENGINE OIL

The 1972 NRC study[26] on particulate polycyclic organic matter (POM) reported findings in the literature that BaP emission increased as the vehicle aged and oil consumption increased from 1,600 mi/qt to 200 mi/qt and that the BaP preferentially concentrated in the crankcase. The report did not list any other POM in used oil.

There are numerous analytic problems in isolating and analyzing for PAHs in used engine oil. Lee et al.[20] sampled oil taken from the oil pans of four randomly selected 4-, 6-, and 8-cylinder automobiles. The qualitative results of high-resolution (capillary) gas chromatography showed peaks for fluorene, phenanthrene, anthracene, 4-5-methylene, 9-methylphenanthrene, fluoranthene, pyrene, 1-methylpyrene, triphenylene, chrysene, BaP, BeP, perylene, and dibenz[ac]anthracene.

Peake and Parker[35] estimated that 500 million gallons of used motor oil is not reclaimed, but is haphazardly discharged into sewers or onto wasteland each year. In analysis of motor oil by gas chromatography-mass spectrometry, they found a predominance of alkyl-substituted aromatic compounds and 11 alkylfluorene isomers. Table 2-3 lists identified compounds and amounts per milliliter of oil, based on the detector response to perdeuteroanthracene.

PETROLEUM AND OTHER FOSSIL-FUEL COMBUSTION FOR HEAT AND POWER GENERATION

The data presented below show the individual PAHs present in emission from several sources of combustion effluents. Compounds known to be carcinogenic are identified in the tabulations of some of the data. A 1980 NRC study[30] recommended that future research should continue to monitor such emission and measure (in a mass-balance study) the contribution of known carcinogenic or mutagenic compounds to the environment. There is a need for specific-site and broad-scale mass-balance studies of the release of PAHs into the atmosphere. The highest PAH emission rates in heat- and power-generation categories given in the 1967 review by Hangebrauck *et al.*[14] were associated with small, domestic, coal-fired furnaces used to heat single-family homes. The emission from oil-burning was generally much lower than that from coal-burning and slightly higher than that from gas-fired units. The emission rates for 10 PAH compounds from under-feed stokers and hand-stoked coal furnaces were higher by several orders of magnitude than those from coal-fired power-plant units, as shown in Tables 2-4 and 2-5, respectively. In Table 2-6, the emission rates for intermediate-sized coal, oil, or gas units using different firing methods show that the under-feed coal-stoker units emit all 10 PAHs at the higher rates.

The EPA Industrial Environment Research Laboratory, Research Triangle Park, N.C. (IERL-RTP), developed the source-assessment sampling system (SASS) train for collection of gaseous, particulate, and volatile exhaust matter. The SASS was used to determine emission data on 74 inorganic trace elements and 21 PAHs in the effluent of 11 industrial coal-stoker-fired boilers. These 11 units represent a wide range of designs, which reportedly have changed very little over the last 20 yr. Three units were spreader stokers with reinjection from the dust collector (Table 2-7), three were without reinjection (Table 2-7), and five were mass-fired over-feed stokers. The study was done by the American Boiler Manufacturers' Association under the joint sponsorship of the U.S. Department of Energy and the EPA by Burlingame *et al.*[6] There were 23 SASS tests conducted, and the emission data were presented in three units: nanograms per joule of energy input, micrograms per dry standard cubic meter of flue-gas samples, and micrograms per kilogram of fuel input. The PAH totals of all the SASS tests were reported, but only the average emission in micrograms per standard cubic meter of effluent and in micrograms per kilogram of fuel is listed in the table.

PAHs FROM COKE PRODUCTION

The EPA's July 1981 report[46] on background information for a proposed standard on coke-oven emission gives a comprehensive overview of emission and locations of coke plants, as well as a cancer risk assessment.

Only some of the literature showing the PAH compounds found in the emission from coke ovens and the relationship to particle size is discussed here.

Lao *et al.*[19] reported the results of sampling emission from coke ovens in the steel industry. Two samples were collected on glass-fiber filters and two on 0.8-μm-pore silver membrane filters, extracted for 24 h in Soxhlet extractors, and measured in GC/MS and GC/FID systems. The results are given in Table 2-8 in micrograms per gram of extract.

Some of the early studies on carcinogenicity of coke-oven effluent (as early as 1875) were reviewed by Hoffmann and Wynder[15] in 1976. The relative risk of developing lung cancer for men employed 5 yr (1951-1955) or more at the full topside of the ovens from exposure to coke-oven effluent was 6.9 times the expected (the prediction was 2.5 times greater than for the general population); an unexpected finding of kidney-cancer incidence 7.5 times greater than in the general population was also reported.

Several reports have discussed the PAH emission from coke ovens in other countries: Norway,[4] Finland,[41] Czechoslovakia,[21] Canada,[37] and Brazil.[24]

In studying concentrations of PAHs on particles of the various sizes, Bjorseth[4] found the greatest amount on the particles of 0.9-3 μm and only about 1% of the total on particles larger than 7 μm (see Table 2-9). The particles were collected at the top of a coke-oven battery, fractionated according to particle size by a Lundgren impactor, and analyzed in a glass-capillary GC/MS system. Miguel and Rubenich[24] gave the concentrations of BaP found on particles in various size ranges (collected with an eight-stage low-pressure impactor that separated by aerodynamic diameter) from an urban automobile traffic tunnel, from ambient air, and from the bench (push side) of a steel-mill coke oven (see Figure 2-1).

COAL MINING

The PAHs found in respirable coal-dust samples were identified as phenanthrene, pyrene, benzo[ghi]fluoranthene, chrysene, perylene, benzoperylene, benzochrysene, and dibenzoperylene. These compounds were more common in coal dust in the mine than in dust from other locations. The OTA report[39] of 1979 stated that the long-term exposure of coal miners to these suspected carcinogenic compounds had not been analyzed. Shultz *et al.*[40] reported finding 13 PAHs in the respirable fraction of mine dusts.

WOOD-BURNING FOR HEAT AND POWER GENERATION

As the prices for home heating with electricity, gas, and oil increase and fuel availability is threatened, many home owners are using wood or coal stoves as a supplement for space heating. Duncan *et al.*[11] estimated an increase of 40,000 wood-burning stoves in 201 counties of the TVA power-distribution area from 1974 to 1976. According to DeAngelis *et al.*,[10] the U.S. Bureau of the Census data showed that 452,000 new homes had fireplaces and that 550,000 wood-burning stoves were shipped by manufacturers in 1975. Owing to the difficulty in achieving controlled combustion in fireplaces and wood- and coal-burning stoves, there is often not an efficient burn; consequently, there is a need for more frequent cleaning of chimneys. Chimney-cleaning equipment is being sold for use by individual homeowners, and the occupation of chimneysweep has become prominent once again. Hazards of exposure to the particulate matter in chimney-cleaning are recognized to be associated with not only skin exposure, but also inhalation. The carcinogenic health hazards associated with chimney-cleaning were reviewed by Bagchi and Zimmerman.[1] The number of housing units burning wood was estimated by DeAngelis et al.,[9] using 1970 U.S. Census of Housing data in conjunction with the 1976 Housing Survey. The state-by-state tabulation showed totals of 912,000 units burning wood as the primary source of heat and 35,467,900 burning wood for auxiliary or aesthetic purposes, with an estimated consumption of 5,122,000 metric tons a year for the primary units and 11,500,000 metric tons for auxiliary or aesthetic units. The range of POM emission from wood stoves and fireplaces was 0.01-0.4 and 0.02-0.04 g/kg, respectively. In 1981, Peters, a coauthor of the above work, estimated the annual emission of POM into the ambient air from primary heating units at 1,383 metric tons, from auxiliary units at 2,376 metric tons, and from fireplaces at 78 metric tons, for a total of 3,837 metric tons.

The NRC report Indoor Pollutants[29] assessed some of the sources of pollutants indoors and their effect on air quality. When wood stoves were in use, the BaP concentration monitored over 24 h indoors was 5 times higher than when stoves were not in use.

In assessing the impact of wood-combustion emission on the environment, the U.S. Department of Energy,[44] in 1979, stated that the major pollutants of concern from residential wood-combustion devices were unburned combustibles, carbon monoxide, particles, and hydrocarbons. Owing to the inefficient combustion in many home-heating units, large quantities of all of them are emitted. According to the 1980 Department of Energy report[45] on health effects of residential wood combustion, the emission from such combustion is a major environmental problem affecting local air quality. POM is the most important group of organic compounds among the noncriteria substances emitted. There is no federal regulation of atmospheric emission applicable to residential space-heating units.

In the preliminary EPA assessment of wood-fired residential combustion equipment, it was stated that the emission of organic substances, including POM, is relatively high, owing to the use of large pieces of fuel, highly resinous fuel, uneven fuel distribution, and hand-feeding in batches. The emphasis on use of wood-burning, air-tight stoves may greatly increase the magnitude of the emission problem. The PAHs emitted from wood-burning in fireplaces and baffled and nonbaffled stoves are shown in Table 2-10.

In a study of modifications of combustion to reduce emission from residential wood-burning, EPA[47] suggested several techniques to reduce the gaseous components in the emission. The following compounds were identified in the emission from wood-burning stoves that burned oak or green pine in either the up-, down-, side-, or high-turbulence-draft delivery systems. The up-draft system had the lowest total-particle and gaseous emission rates.

Naphthalene
Acenaphthene
Acenaphthylene
Fluorene
Phenanthrene
Anthracene
Methylanthracene/methylfluoranthenes
Fluoranthene
Pyrene
Methylpyrenes/methylfluoranthenes
Benz[a]anthracene
Chrysene
Methylchrysenes
Dimethylbenz[a]anthracene
Benzofluoranthenes
Benzo[e]pyrene
Benzo[a]pyrene
Perylene
Indeno[1,2,3-cd]pyrene
Benzo[ghi]perylene
Coronene
Dibenzo[ah]pyrene

PAH emission rates under controlled burn conditions were characterized by Hubble *et al.*,[16] and their results are shown in Table 2-11. The relationship of ambient air concentrations of PAHs to the sources of wood-burning has been studied by several investigators. In some circumstances, wood-burning can be the largest combustion source for various atmospheric pollutants in urban areas. For example, the contribution of wood-burning emission products to atmospheric PAHs in Telluride, Colorado, has been estimated by Murphy *et al.*[25] Telluride is a small community in a valley with poor ventilation and with large temperature inversions. It depends heavily on wood-burning as a residential heating source and has only light automobile traffic. In this community in 1980, the BaP concentration in the air reached 7.4 ng/m^3, which exceeds several times over that which is found in a number of U.S. metropolitan areas, such as Los Angeles, and Telluride is not unique in this respect.

The mechanisms of PAH formation during the combustion of wood are poorly understood. It is known that wood contains substantial amounts of alkylbenzene derivatives, which contribute to the formation of the PAHs. The latter reactions depend markedly on temperature: no important formation of hydrocarbons occurs below 450°C. Approximately 75 organic compounds have been identified in flue-gas samples of the identified organic substances, and the PAHs make up about 35% of the mass. The PAHs that are produced during the pyrolysis of wood and are found in the smoke include anthracene, phenanthrene, dibenz[aj]anthracene, dibenz[ah]anthracene, fluoranthene, benzo[ghi]fluoranthene, benzo[b]fluoranthene, benzo[c]phenanthrene, benzo[ghi]perylene, pyrene, BaP, BeP, 3-methylcholanthrene, dibenzo[cg]carbazole, dibenzo[ai]carbazole, cyclopenta[cd]pyrene, and some methylated substances.

A detailed study of the effects of wood type, degree of seasoning (e.g., the moisture content), and type of wood stove on the emission of PAHs in smoke has been conducted.[36] A fireplace, a baffled and a nonbaffled wood stove, and seasoned (4-5% moisture) and green (27-30% moisture) oak and pine were used. The results are indicated in Table 2-13. Wood smoke generated in a fireplace contained much less PAH than that formed in wood stoves. No difference in relative distribution of particular PAHs was noted when green and seasoned wood were burned in the fireplace (data not shown). Furthermore, neither wood type nor extent of baffling significantly influenced the pattern of PAH emission in the wood smoke.

Peters[36] has compared the emission of PAHs from several residential combustion sources as a function of thermal efficiency (Table 2-14). Wood-fired heating resulted in a much higher output of PAHs than did coal-, oil-, or gas-fired furnaces; i.e., the mass of PAHs emitted per joule was 10, 5,000, and 30,000 times greater, respectively.

REFUSE BURNING AND INCINERATION

Before the passage of the Solid Waste Disposal Act in 1965, numerous municipal dumps practiced open or uncontrolled burning throughout the United States. Owing to the standards established under this Act, most of the large incinerators then in operation were shut down, largely because upgrading them would have been expensive. Congress passed the Resource Recovery Act in 1970 and the Resource Conservation and Recovery Act in 1976. As a consequence of the passage of these acts, numerous demonstration projects for solid-waste disposal and energy and resource recovery were initiated. The concluding remarks of the 1981 NRC report The Recovery of Energy and Materials from Solid Waste[27] stated "that the technologies for energy recovery were still under development and that the most highly developed and least risky was mass burning, but that other technologies were being tested."

The solid waste from residential, commercial, and institutional sources amounted to 130 million metric tons in 1976 and is projected to increase to 180 million tons by 1985.[48]

In EPA's 1971 review[42] of the literature on municipal incineration, the findings of Hangebrauck *et al.*[14] (Table 2-15) showed the emission of 10 PAHs from municipal and commercial units burning wastes from households, grocery stores, and restaurants. In 1976, Davies *et al.*[8] reported that the measured emission of PAHs was similar to that found by Hangebrauck. They used a modern, continuous-feed municipal refuse incinerator rated at 9.14 tons of refuse per hour with a water-spray cooling tower (cooled to 250-300°C) and an electrostatic precipitator in the flue-gas stream. The analytic results on samples collected after the electrostatic precipitator are given in Table 2-16.

The findings by several investigators on the emission of chlorinated dibenzo-*p*-dioxins in waste incineration have caused some concern about the human-health implications of this group of compounds, but discussion of dioxin emission is beyond the scope of this report.

METAL PROCESSING

The stack gases from a smelter processing lead from batteries were sampled. The PAHs and their concentrations found in four samples are shown in Table 2-17. The polymeric organic battery casings were included in the process and presumed to be the contributing source of the organic emission. Lao and Thomas[18] identified several PAH compounds in the particles collected on glass-fiber filters in the exhaust flue from the "pot room" of a nonferrous-metal production room. Particles from iron foundries in Finland were analyzed for PAHs by Schimberg,[38] and the following were identified: phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]fluorene, benzo[c]phenanthrene, benzofluoranthenes, BeP, BaP, perylene, *o*-phenylenepyrene, dibenzanthracenes, benzochrysenes, and benzo[ghi]perylene.

NATURAL SOURCES

The mechanism whereby complex mixtures of PAHs occur in natural deposits of peat, coal, crude oil, and shale oil is unknown. Neff[33] discussed the sources of PAHs in the aquatic environment and their possible biosynthesis by bacteria, yeasts, and higher plants. Although some publications apparently provide evidence of biosynthesis, others refute it. Until relatively recently, it was assumed that PAHs are formed only by pyrolysis of organic material. However, the finding of 1-methyl-7-isopropylphenanthrene (retene) in pine tar and the isolation of PAHs directly from plant material have helped to keep alive the uncertainty of PAH biosynthesis in plants.

FOREST FIRES

Forest fires are sporadic and sometimes uncontrollable occurrences that apparently contribute significantly to the PAHs found in the atmosphere. Some laboratory data are given below on the amount of PAH emission. The discussion in Chapter 3 of physical removal of PAHs from the atmosphere mentions forest fires as a source of atmospheric PAHs. This source is mentioned here, because it has the potential for being a significant contributor; however, data are insufficient for an assessment of the impact on atmospheric quality. The 1976 NRC report Air Quality and Smoke from Urban and Forest Fires[28] described "prescribed forest fires" as fires set to reduce the amount of secondary plant or roughage undergrowth in the hope of reducing the incidence of wild fires, for disease control, and for other management purposes. In the 1976 report, it was noted that wild forest fires consume about 3 times as much fuel as prescribed fires and produce about 3 times as much particulate matter per ton of fuel burned as prescribed fires. On the average, prescribed fires consume 3 tons of fuel per acre (range, 1-10 tons/acre) and wild fires 9 tons/acre (range, 1-50 tons/acre).

Emission from forest fires varies widely, owing to the variety of fuels, fire type (heading fires, with the fire line moving with the wind, or backing fires, with the fire line moving against the wind), fire intensity, and combustion phase (flaming vs. smoldering). The results of a screening experiment reported by McMahon and Tsoukalas,[23] using slash pine needle litter as fuel, are shown in Table 2-18. The PAHs present in the particles show that heading fires produce higher total amounts of particles, but smaller total amounts of PAHs.

AQUATIC ENVIRONMENTS (FRESHWATER AND MARINE)

In its twenty-fifth report to Congress, the Committee on Governmental Operations[43] considered groundwater destruction one of the most serious environmental problems of the 1980s. It estimated that 50 billion gallons of water are placed in industrial surface-water impoundments throughout the country each year. The report stated that tens of millions of Americans obtain their water from private wells, because no public water supply is available.

A high percentage of PAHs is removed from municipal water by flocculation and sedimentation of suspended particulate matter. The chlorination process removes a large percentage of residual PAH contamination (the amounts removed by this process are discussed elsewhere in this report). We do not have national survey information on the incidence of well-water contamination by PAHs, and this route of human exposure has not been assessed. But it is of potential importance, in that most well-owners do not treat the water before use, on the assumption that it is uncontaminated. Owing to the large number of industrial wastewater impoundments that are unlined (no plastic liner) and are directly over groundwater sources, there is a need for investigation to determine whether there is PAH contamination. (See the section of this report on human exposure for discussion of PAHs in drinking water.)

In the comprehensive review by Neff[33] on PAHs in the aquatic environment, the following sources of pollution were listed: industrial and domestic sewage effluent, surface runoff from land, deposition of airborne particles, and spillage of petroleum and petroleum products into water bodies. Specific PAHs from these sources are discussed earlier in this section; but Neff's work includes more detail on the effects on the aquatic environment. The 1975 NRC report *Assessing Potential Ocean Pollutants*[32] discussed the effects of several sources of pollution, e.g., ocean discharge of dredge spoil, municipal sewage sludge, petroleum discharge, and spills. Although that report did not discuss the effects specifically of PAHs, the sources of pollution described in it have been identified as sources of PAHs. An NRC committee is in the process of reassessing the pollutants and their effects on the marine environment.

In 1974, owing to the increasing amounts of petroleum being transported over the world's waterways, the need for a comprehensive, international marine environmental monitoring assessment program led to a symposium and workshop on marine pollution monitoring held at the National Bureau of Standards in Gaithersburg, Maryland. At the symposium, deep concern was expressed about the amount of petroleum pollution observed in the marine environment and its effects and ultimate fate. Among those expressing concern were representative of several national and international organizations with interests and oversight for pollution problems in the marine environment, such as the Special

Committee on Problems of the Environment of the International Council of Scientific Unions, the International Oceanographic Commission, the Intergovernmental Working Group on the Global Environmental Monitoring System (the EARTHWATCH program), the Integrated Global Ocean Station System, and the Experts on Scientific Aspects of Marine Pollution (supported by the United Nations Environment Program). Out of concern like that expressed at the 1974 symposium, there arose the Mussel Watch that uses mollusks (mussels, clams, and oysters) as biologic monitors of aquatic pollution. The 1980 NRC report The International Mussel Watch[31] described the program and gave the two general aims of the "watch": to produce information on the contamination of coastal ecosystems and food resources and global data on the abundance of anthropogenic contaminants.

TABLE 2-1

Polycyclic Aromatic Hydrocarbons in Coal, Oil-Shale, and Petroleum Isolates[a]

	Relative Peak Values[b]			
PAH	Coal Synthoil C	Coal Syncrude D	Shale B	Petroleum Mix A
Fluorene	5.3	9.9	2.2	3.9
9-Methylfluorene	2.0	1.4	0.7	2.6
1-Methylfluorene	6.0	<4.7	3.2	5.6
Phenanthrene	20.4	12.0	4.0	5.3
Anthracene	--	4.1	1.8	T
2-Methylanthracene	2.4	3.0	1.1	--
1-Methylphenanthrene	<1.2	<5.1	3.6	8.1
9-Methylanthracene	--	--	T	--
Fluoranthene	<1.9	<3.7	1.6	0.9
Pyrene	35.0	14.2	3.7	4.3
Benzo[a]fluorene	2.5	2.1	1.1	2.1
Benzo[b]fluorene	3.4	<1.5	1.2	2.2
1-Methylpyrene	<8.0	<6.0	1.6	1.6
Benzo[c]phenanthrene	<0.6	<2.2	1.3	3.1
Benzo[ghi]fluoranthene	3.2	--	--	0.3
Benz[a]anthracene	<2.2	T	1.0	T
Chrysene	2.5	<1.5	1.5	2.6
Benzo[b, j, and/or k]fluoranthene	<1.3	<0.5	0.5	0.2
Benzo[e]pyrene	1.3	<1.2	0.3	0.3
Benzo[a]pyrene	<1.2	<0.5	0.4	0.2
Perylene	T	<0.6	T	0.7
Dibenz[ac and/or ah]anthracene, indeno[1,2,3-cd]-pyrene[c]				
Picene[c]				
3-Methylcholanthrene	<1.2	<1.3	T	0.70
o-Phenylenepyrene	2.6	1.1	--	--
Benzo[ghi]perylene	6.6	4.3	--	--
Anthanthrene	<0.8	T	--	--

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[a] Adapted from Guerin *et al.*[12]

[b] Relative peak values show a relationship of amounts present. Dash indicates none detected. T indicates trace quantity detected. < indicates known to contain co-eluting species.

[c] Peaks identified, but relative values not sought.

TABLE 2-2

PAHs Resulting from Gasification of Western Coal, Coal Blend, Wood, and Peat[a]

Compound	Concentration, μg/g of feed stock North Dakota Lignite	Wyoming Smith-Roland[b]	Montana Rosebud Coal	Ill.#6 & CaCO Blend[c]	Ill.#6 & Wood Pellets[d]	N. C. Peat Pellets[e]
Naphthalene	1,520	899	1,430	878	236	2,480
Acenaphthylene	322	182	234	278	53.6	343
Fluorene	137	82.2	45.5	117	28.9	101
Phenanthrene	443	394	375	412	94.0	542
Anthracene	81.4	65.9	49.0	224	32.7	108
Pyrene	221	253	405	230	31.3	395
Benz[a]-anthracene	35.7	48.3	49.0	98.2	6.3	67.0
Chrysene	18.6	32.1	30.9	69.0	3.8	33.9
Benzo[b]-fluoranthene	22.9	32.1	54.3	43.9	3.1	35.8
Benzo[k]-fluoranthene	7.1	7.9	1.2	23.0	2.1	17.9
Benzo[a]pyrene	17.1	23.7	52.5	37.6	3.8	34.9
Indeno[1,2,3-cd]pyrene	4.7	2.2	4.4	4.2	0.7	0.9
Dibenz[ah]-anthracene	5.2	11.0	33.1	16.7	0.7	11.3
Benzo[ghi]-perylene	2.9	4.8	34.0	10.4	0.7	7.5

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[a] Adapted from Nichols *et al*.[34]

[b] Subbituminous coal.

[c] 70% coal, 30% limestone.

[d] 79% coal, 21% hardwood pellets.

[e] North Carolina coastal peat (19% moisture).

TABLE 2-3

Polycyclic Aromatic Hydrocarbons in Used Motor Oil Identified by Gas Chromatography-Mass Spectrometry[a]

Compound	µg/ml oil[b]
Methylbiphenyl	0.74
Methylbiphenyl	0.36
Methylbiphenyl	0.26
Fluorene	1.47
Methylbiphenyl	0.42
Methylbiphenyl	0.18
Methylbiphenyl	0.09
Methylfluorene	0.10
Methylfluorene	1.19
Methylfluorene	0.08
Phenanthrene	7.80
Deuterated anthracene[c]	0.50
Methylfluorene	0.08
Dimethylfluorene[d]	0.58
	0.10
Anthracene	0.33
Dimethylfluorene[d]	0.61
Methylphenanthrene	2.63
Methylphenanthrene	3.62
Methylphenanthrene	2.95
Trimethylfluorene[e]	0.12
Methylphenanthrene	2.44
Trimethylfluorene[e]	0.29
Trimethylfluorene[e]	0.36
Phenylnaphthalene	0.90
Trimethylfluorene[e]	0.15
Trimethylfluorene[e]	0.18
Dimethylphenanthrene[d]	0.22
Dimethylphenanthrene[d]	0.16
Dimethylphenanthrene[d]	0.75
Methylanthracene	0.09
Dimethylphenanthrene[d]	2.45
Dimethylphenanthrene[d]	4.21
Methylanthracene	0.28
Dimethylphenanthrene[d]	2.80
Fluoranthene	4.36
Methylanthracene	0.21
	0.08
Ethylcyclopenta[def]phenanthrene[c]	0.79
Ethylcyclopenta[def]phenanthrene[c]	0.46
	0.10
Trimethylphenanthrene[e]	0.10
Pyrene	6.69

Table 2-3 (cont.)

Compound	μg/ml oil[b]
Ethylcyclopenta[def]phenanthrene[d]	0.17
	0.39
Trimethylphenanthrene[e]	0.08
Trimethylphenanthrene[e]	0.29
Terphenyl	0.12
Trimethylphenanthrene[e]	1.32
Dimethylanthracene[d]	0.08
Dimethylanthracene[d]	0.10
Trimethylphenanthrene[e]	2.72
Trimethylphenanthrene[e]	0.48
Dihydromethylpyrene[f]	0.13
Trimethylphenanthrene[e]	1.16
Benzo[a]fluorene	0.93
Benzo[b]fluorene	1.38
Benzo[c]fluorene	0.44
Methylpyrene[f]	1.19
Trimethylanthracene[e]	0.51
Dihydromethylpyrene[f]	0.32
Methylpyrene[f]	1.14
Methylpyrene[f]	1.14
Methylpyrene[f]	0.78
Diethylphenanthrene[g]	0.18
Dimethylpyrene[f]	0.13
Diethylphenanthrene[g]	0.14
Dimethylpyrene[d]	0.70
Diethylphenanthrene[g]	0.34
Dimethylpyrene	0.28
Dimethylpyrene	0.33
Benzo[c]phenanthrene	0.12
Dimethylpyrene[d,f]	0.27
Ethylmethylpyrene[d,f]	0.14
Benzo[a]anthracene	0.87
	0.22
Chrysene + triphenylene	2.48
Cyclopenta[cd]pyrene	0.78
Methylbenzo[a]anthracene[h]	1.68
Methylbenzo[mno]fluoranthene[i]	0.15
Methylbenzo[a]anthracene[h]	0.26
Methylbenzo[a]anthracene[h]	0.23
Methylbenzo[mno]fluoranthene[i]	0.15
Methylbenzo[a]anthracene[h]	0.26
Methylbenzo[a]anthracene[h]	0.15
Methylbenzo[a]anthracene[h]	0.28
Ethylbenzo[a]anthracene	0.44
Ethylbenzo[a]anthracene	0.21
Benzo[k]fluoranthene	1.44
Benzo[e]pyrene	1.74

Table 2-3 (cont.)

Compound	μg/ml oil[b]
Benzo[a]pyrene	0.36
Perylene	0.13
Methylbenzofluoranthene	0.18
Methylbenzopyrene[j]	0.41
	0.32
Benzo[ghi]perylene	1.67

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[a] Reprinted with permission from White et al.;[49] copyright Ann Arbor Science Publishers, Inc.

[b] μg/ml of oil based on the response of deuterated anthracene.

[c] Internal standard.

[d] Could be ethyl- or dimethyl-.

[e] Could be ethylmethyl-, trimethyl-, or propyl-.

[f] Could be a pyrene or fluoranthene.

[g] Could be diethyl-, ethyldimethyl-, tetramethyl-, methylpropyl-, or butyl-.

[h] Could be a derivative of chrysene, triphenylene, benzo[c]phenanthrene or benz[a]anthracene.

[i] Could be a derivative of benzo[mno]fluoranthene or cyclopenta[cd]pyrene.

[j] Compounds with molecular weight 276 can be any of the following: indeno[1,2,3-cd]pyrene, indeno[1,2,3-cd]fluoranthene, cyclopenta[cd]-perylene, phenanthro[10,-1,2,3-cdef]fluorene, acenaphth[1,2-a]-acenaphthylene, dibenzo[b,mno]fluoranthene, dibenzo[e,mno]fluoranthene, and dibenzo[f,mno]fluoranthene. Further possibilities are the benzo derivatives of cyclopenta[cd]pyrene and cyclopenta[cd]fluoranthene.

TABLE 2-4

PAH Emission Summary: Heat Generation by Coal-Fired Residential Furnaces[a]

Sample No.	Firing Method	Benzene-soluble Organics g per 10^6 Btu	Group 1										Group 2		
			BaP												
			µg/g b sol.	µg per 1,000 m³ c	µg per lb fuel	BaP	P	BeP	Per	BghiP	Anth	Cor	A	Phen	Fluor
						µg per million Btu heat input									
188-19	Under-feed stokers	2.4	1,600	3,400	52	3,800	7,700	5,440		580		1,200		29,000	47,000
34		47	1,400	40,000	900	65,000	300,000	39,000	7,900	61,000	6,100	4,100	70,000	610,000	330,000
36		73	1,100	44,000	1,200	81,000	290,000	59,000	4,800	58,050	3,000		48,000	350,000	150,000
59		20	3,400	18,000	930	67,000	160,000	55,000	5,500	39,000	1,300	3,400	14,000	170,000	120,000
60		8.7	990	2,200	120	8,600	45,000	7,700	430	6,300			1,300	51,000	76,000
20	Hand-stoked	91	3,900	340,000	6,000	400,000	600,000	100,000	60,000	300,000	90,000	30,000	400,000	1,000,000	1,000,000
57		170	10,400	690,000	25,000	1,700,000	2,700,000	870,000	220,000	1,400,000	270,000	49,000	1,100,000	2,300,000	4,300,000
58		350	9,400	1,500,000	46,000	3,300,000	9,100,000	1,500,000	350,000	2,200,000	490,000	97,000	2,900,000	7,300,000	11,000,000

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[a] Reprinted from Hangebrauck *et al.*[14] A blank indicates that the compound was not detected in the sample.

[b] Micrograms per gram of benzene-soluble organic substances.

[c] Micrograms per 1,000 m³ of flue gas at standard conditions (70°F, 1 atm).

TABLE 2-5

PAH Emission Summary: Heat Generation by Coal-Fired Power Plants[a]

Sample No.[b]	Type of Unit	Sampling Point[c]	Benzene-soluble Organics g per 10^6 Btu	Group 1										Group 2		
				BaP[d]												
				μg/g soluble[e]	μg per 1,000 m^3[f]	μg per lb fuel	BaP μg per million Btu heat input	P	BeP	Per	BghiP	Anth	Cor	A	Phen	Fluor
IRB-22		B	3.0	16	110	0.38	49	150			45	16				
26	Pulverized coal (vertically fired, dry-bottom furnace)	B	0.90	22	50	0.26	22	136								190
25		A	1.7	11	42	0.23	19	190								250
27		A	2.0	9.3	42	0.22	19	120								180
28		B	1.6	39	130	0.66	56	100	33		19					320
29		A	1.4	30	120	0.63	55	230	41							410
30		B	1.3	330	930	3.3	660	860	250	66	160	35	4.7	110	820	1,700
31		A	1.2	110	270	1.3	130	74	79	73	82					84
42	Pulverized coal (front-wall-fired, dry-bottom furnace)	A	0.36	48	39	0.21	17	200	55		16				300	160
44		A	0.62	33	48	0.28	21	160								13
61 & 62	Pulverized coal (tangentially fired, dry-bottom furnace)	A	0.65	230	320	1.7	140	160	86	71	150	4.9	7.1		32	300
63	Pulverized coal (opposed-, downward-inclined burners; wet-bottom furnace)	A	1.3	110	330	1.9	140	130	420		1,100		93			210
64		A	1.1	21	57	0.29	22	51	110		190		19			65
65		A	1.0	21	46	0.28	21	39	72		150		8.1			33

Table 2-3 (continued)

Sample No.[b]	Type of Unit	Sampling Point[c]	Benzene-soluble Organics g per 10^6 Btu	Group 1 BaP[d] μg/g soluble[e]	μg per 1,000 m³[f]	μg per lb fuel	BaP	P	BeP	Per	BghiP	Anth	Cor	Group 2 A	Phen	Fl'nt
							μg per million Btu heat input									
69	Crushed coal (cyclone-fired, wet-bottom furnace)	A	2.1	175	730	5.2	370	1,000	680	34	360					110
70		A	0.92	83	170	1.1	76	250	110		36		11		370	46
71	Spreader stoker (traveling grate)	A	1.1	22	58	0.31	24	59	61				9.5			59
72		A	1.8	8.5	36	0.19	15	32								32
73		A	1.4	11	33	0.19	15	21								21

[a] Reprinted from Hangebrauck et al.[14]

[b] Additional filter used before the bubblers in the sampling train.

[c] B: sampling point before fly ash collector; A: after fly ash collector.

[d] "Less than" values for benzo[a]pyrene were calculated for samples having concentrations below the limit of quantitative determination (approximately 0.6 μg per sample). Similar calculations were not included for the other PAHs (indicated by blanks in the table).

[e] Micrograms per gram of benzene-soluble organic substances.

[f] Micrograms per 1,000 m³ of flue gas at standard conditions (70°F, 1 atm).

TABLE 2-6

PAH Emission Summary: Heat Generation by Intermediate Coal-Fired Units
and Intermediate and Small Oil- and Gas-Fired Units[a]

			Benzene soluble Organics	Group 1										Group 2		
				BaP	BaP[b]			P	BeP	Per	B(ghi)P	Anth	Cor	A	Phen	Fluor
Sample No.	Fuel	Firing Method	mg per 10^6 Btu	µg/g d en[c]	µg per 1,000 m^3 [d]	µg per lb fuel	µg per million Btu heat input									
123-6	Coal	Pulverized	2.9	11	75	0.43	32	240	92					370		550
7		Chain-grate stoker	1.9	19	71	0.44	37	390	130							680
5		Spreader stoker	5.4	4.7	49	0.35	26	590	350				26			360
4		Underfeed	3.0	3,400	7,900	146	10,000	16,000	7,900	1,800	4,300	290	330	850	10,000	38,000
16		stokers	6.0	29	61	1.6	120	1,700	230						1,800	3,300
8	Oil	Steam-	1.4	<11	<38	<0.3	<20	49								56
12		atomized	3.3	15	40	0.89	47	300							1,800	270
10		Low-pressure air-atomized	14	65	1,900	10	900	6,100			500		2,100	3,900	3,500	1,900
17		Pressure-	8.1	<4.6	<26	<0.9	<40	1,800							8,900	5,000
13		atomized	3.6	<17	<27	<1	<60	15								76
15		Vaporized	3.5	<33	<34	<2	100	1,200								15,000
11	Gas	Premix	1.1	<17	<29	<0.4	<20	160					14			100
9		burners	1.2	170	350	4.6	200	18,000	490		1,800	200	5,300			2,900
18			0.95	<21	<23	<0.5	<20	170								320
16			0.65	<35	<30	<0.6	<20	120	18						77	110
47			5.2	51	71	6.1	270	16,000	1,500		2,300	73	830			8,000

[a] Reprinted from Hangebrauck *et al.*[14]

[b] "Less than" values for benzo[a]pyrene were calculated for samples having concentrations below the limit of quantitative determination (approximately 0.6 μg per sample). Similar calculations were not included for the other PAHs (indicated by blanks in the table).

[c] Micrograms per gram of benzene-soluble organic substances.

[d] Micrograms per 1,000 m^3 of flue gas at standard conditions (70°F, 1 atm).

TABLE 2-7

Summary of Average PAH Emission from Coal-Stoker-Fired Boilers With and Without Reinjection from the Stoker Dust Collector[a]

Compound	Concentration[b]			
	Reinjected		Without Reinjection	
	µg/dry SCM[c]	µg/kg of fuel	µg/dry SCM[c]	µg/kg of fuel
Phenanthrene	9.60	66.0	9.40	76.0
Anthracene	0.29	2.0	8.50	68.0
Methylanthracenes/ phenanthrenes	0.66	--	0.84	--
Fluoranthene	2.50	--	8.60	--
Pyrene	0.86	--	0.37	--
Methylpyrene/ fluoranthene	0.21	--	0.12	--
Benzo[c]phenanthrene	0.069	--	0.093	--
Benz[a]anthracene	0.10	--	0.07	--
Chrysene	0.15	--	0.12	--
Methylchrysenes	0.051	--	0.059	--
Dimethylbenzanthracenes	0.028	--	--	--
Benzofluoranthenes	0.13	--	0.09	--
Benzo[e]pyrene	0.044	--	0.047	--
Benzo[a]pyrene	0.039	--	0.043	--
Perylene	0.036	--	0.043	--
Indeno[1,2,3-cd]-pyrene	0.055	--	0.061	--
Benzo[ghi]perylene	0.025	--	0.093	--
3-Methylcholanthrene	--	--	0.028	--
Dibenzopyrene	--	--	0.047	--
Coronene	--	--	0.029	--

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[a] Adapted from Burlingame *et al.*[6] Average of all emission from cyclone filters, condensates, impingers, and XAD-2 absorbent resins. Note: The analytic results are accurate to within a factor of 3.

[b] Dashes indicate not calculated.

[c] µg of emission component per standard cubic meter (SCM) of effluent.

TABLE 2-8

PAH Concentrations in Coke-Oven Emission[a]

Compound	Sample No. 1[b]		Sample No. 2[c]		Sample No. 3[d]		Sample No. 4[e]	
	Peak No.	Concentration, µg/g of sample	Peak No.	Concentration, µg/g of sample	Peak No.	Concentration, µg/g of sample	Peak No.	Concentration, µg/g of sample
Octahydrophenanthrene	1	31.85	—	—	—	—	—	—
Octahydroanthracene	1a	29.89	1	8.77	1	29.98	2	70.31
Dihydrofluorene	2	30.31	2	13.62	1a	15.68	2a	18.26
Dihydrofluorene	2a	18.76	—	—	—	—	2b	23.20
Benzindene	3	106.73	2a	6.72	2	58.15	2c	20.81
Fluorene	4, 4a	271.52	3	20.86	3	19.29	2d	15.37
Dihydrophenanthrene	4b, 5	386.98	4	79.55	4	316.49	3	325.83
Dihydroanthracene	6	168.88	5	21.92	5	101.68	4	232.54
2-Methylfluorene	7	98.71	6	9.06	5a	8.87	5	40.28
1-Methylfluorene	8	73.46	7	24.35	6	44.01		
9-Methylfluorene	9	44.32	8	11.81	7	6.71	6	102.83
Methylfluorene	10	87.84	9	10.77	—	—	6a	10.38
Benzoquinoline	11	77.74	10	8.34	8	31.41	7	79.77
Acridine	12, 12a	85.98	11	32.44	9	74.98	8	172.79
Phenanthrene	13	2,828.54	12	163.53	11	458.80	9	636.98
Anthracene	14	942.85	13	46.44	11a	305.89	9a	500.36
Fluorine carbonitrile	15	180.29	14	16.76	11b	32.55	9b	11.47
Methylphenanthrene	16	1,023.41	15	44.67	12	130.22	10	283.92
Methylanthracene	17	1,692.26	16	85.30	13	258.93	11	573.25
Ethylphenanthrene	18	1,578.60	17	58.04	14	202.97	12	197.39
Ethylanthracene	19	1,096.71	18	49.19	15	269.86	13	1,473.68
Octahydrofluoranthrene and octahydropyrene	20	280.42	19	11.06	16	64.36	13a	44.53
Dihydrofluoranthene	21	115.07	20	73.57	17	64.87	13b	31.86
Dihydropyrene	22	575.06	21	36.65	18	52.29	14	484.25
Fluoranthene	23	5,979.74	22	269.74	19	451.95	15	1,097.82
Dihydrobenzo[a]fluorene	24	791.41	23	24.03	—	—	—	—
Dihydrobenzo[b]fluorene and dihydrobenzo[c]-fluorene	25	213.53	24	28.77	20	99.07	16	46.38

Table 2-8 (continued)

Compound	Sample No. 1[b] Peak No.	Sample No. 1[b] Concentration, µg/g of sample	Sample No. 2[c] Peak No.	Sample No. 2[c] Concentration, µg/g of sample	Sample No. 3[d] Peak No.	Sample No. 3[d] Concentration, µg/g of sample	Sample No. 4[e] Peak No.	Sample No. 4[e] Concentration, µg/g of sample
Pyrene	26	4,627.33	25	206.35	21	472.09	17	1,446.64
Benzo[a]fluorene	27	971.18	26	87.42	22	62.97	17a	37.21
Benzo[b]fluorene	28	109.45	27	16.70			17b	22.63
Benzo[c]fluorene	29	627.02	28	38.96	23	290.03	17c	98.47
Methylfluoranthene	30	1,817.37	29	124.73	24	126.03	18	101.56
Methylfluoranthene	31	390.94	30	21.87	25	179.06	18a	36.83
Methylpyrene	32	1,016.76	31	31.12	26	97.80	19	120.66
Methylpyrene	33	856.91	32	106.34	27	233.37	20	175.42
Benzo[c]phenanthrene	34	220.45	33	82.70	28	1,510.34	21	2,156.14
Benzo[ghi]fluoranthene	35	677.35	34	164.25	29	201.74	21a	151.88
Dihydrobenz[a]anthracene, dihydrochrysene, and dihydrotriphenylene	36	383.03	34a	54.75	29a	101.78	22	271.38
Benz[a]anthracene	37	2,760.45	35	105.15	30	5,509.43	23	2,673.63
Chrysene and triphenylene	38	4,202.02	36	119.06				
Dihydromethylbenz[a]anthracene, dihydromethylchrysene, and dihydromethyltriphenylene	39	841.67	37	93.38	30a	247.80	23a	86.35
Methylbenz[a]anthracene	40	159.33	38	22.36	31	1,015.70	24	1,659.72
Methyltriphenylene	41	463.99	39	40.81	32	371.97	25	369.18
Methylchrysene	42	1,151.61	40	107.50	33	193.68	26	449.19
Dihydromethylbenzo[k and b]fluoranthene and dihydromethylbenzo[a and e]pyrene	43	434.38	40a	8.63	34, 35	185.08	26a	137.26
Dimethylbenz[a]anthracene, dimethyltriphenylene, and dimethylchrysene	44, 44a	266.35	41	46.73	36 37 38	174.71 62.25 30.16	27 28	160.89 431.70
Benzo[j]fluoranthene	45	176.92	42	18.66	39	80.06	29	285.34
Benzo[k]fluoranthene and benzo[b]fluoranthene	46	3,930.34	43	155.03	40	2,170.92	30	2,556.98

Table 2-8 (continued)

Compound	Sample No. 1[b]		Sample No. 2[c]		Sample No. 3[d]		Sample No. 4[e]	
	Peak No.	Concentration, µg/g of sample	Peak No.	Concentration, µg/g of sample	Peak No.	Concentration, µg/g of sample	Peak No.	Concentration, µg/g of sample
Methylbenzo[k]fluoranthene and methylbenzo[b]fluoranthene	47	735.95	44	33.05	41	430.05	31	492.13
Benzo[e]pyrene	48	103.86	—	—	—	—	—	—
Benzo[a]pyrene	48a	2,630.92	45	122.15	42	2,007.75	32	2,297.83
Perylene	49	702.12	46	22.07	43	616.85	33	698.44
Methylbenzo[a]pyrene	50	339.85	47	5.88	44	344.12	34	267.96
Dimethylbenzo[k]-fluoranthene and dimethylbenzo[b]-fluoranthene	51	116.74	—	—	45	73.13	—	—
Dimethylbenzo[a]pyrene	52	82.60	—	—	46	70.18	—	—
Dibenzanthracene	53	123.66	—	—	47	84.52	—	—
o-Phenylenepyrene	54	101.54	—	—	—	—	—	—
Benzo[ghi]perylene and anthanthrene	55	72.35	—	—	—	—	—	—
Methyldibenzanthracene	56	89.04	—	—	—	—	—	—
Methylbenzo[ghi]perylene	57	36.79	—	—	—	—	—	—
Coronene	58	864.55	—	—	51	833.30	35	766.58
Dibenzopyrene	59	693.21	—	—	52	587.05	36	493.27

[a] Reprinted with permission from Lao *et al*.[19]

[b] Glass-fiber filter No. 1: total weight of material collected, 12.59 mg; total volume of solvent extract, 1 ml; injected sample size, 10 µl.

[c] Glass-fiber filter No. 2: total weight of material collected, 39.67 mg; total volume of solvent extract, 1.0 ml; injected sample size, 10 µl.

[d] Silver membrane filter No. 1: total weight of material collected, 5.75 mg; total volume of solvent extract, 0.6 ml; injected sample size, 6 µl.

[e] Silver membrane filter No. 2: total weight of material collected, 1.96 mg; total volume of solvent extract, 1.0 ml; injected sample size, 10 µl.

TABLE 2-9

PAHs on Particles from Coke Ovens as a Function of Particle Size[a]

Size	>15 µm	7-15 µm	3-7 µm	0.9-3 µm	<0.9 µm
PAH	µg	µg	µg	µg	µg
Phenanthrene	0.5	0.5	0.8	2.0	b
Anthracene	0.3	0.2	0.6	0.7	b
Methylphenanthrene/ methylanthracene[c]	d	d	0.4	1.3	d
Fluoranthene	d	d	4.2	10.7	2.0
Dihydrobenzo[ab]-fluorene	d	d	0.6	2.1	d
Pyrene	d	d	4.3	12.3	1.3
Benzo[a]fluorene	d	d	3.5	9.1	0.5
Benzo[b]fluorene	d	d	2.6	7.2	0.3
Benzo[c]phenanthrene	d	d	2.0	5.2	0.8
Benz[a]anthracene	0.03	0.08	3.3	9.0	4.0
Chrysene/triphenylene	0.06	0.10	5.8	13.2	1.8
Benzo[bjk]fluoranthene	0.05	0.40	4.8	10.6	7.2
Benzo[e]pyrene	0.03	0.14	3.4	8.9	0.5
Benzo[a]pyrene	0.02	0.12	5.2	12.1	1.4
Perylene	d	d	1.3	2.9	d
o-Phenylenepyrene	d	d	3.8	7.2	0.5
Benzo[ghi]perylene	d	d	5.2	7.8	1.3
Anthanthrene	d	d	1.2	3.2	0.3
Coronene	d	d	5.9	6.3	0.5
Dibenzopyrene	d	d	0.9	1.5	0.3

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[a] Reprinted with permission from Bjorseth;[4] copyright Ann Arbor Science Publishers, Inc.

[b] Detectable.

[c] Isomer not determined.

[d] Not detected.

TABLE 2-10

POM Emission (g/kg)[a]

Compounds	Fireplace			Baffled Stove		Nonbaffled Stove		
	Seasoned Oak		Green Pine	Seasoned Oak	Seasoned Pine	Seasoned Oak	Green Pine	
	POM Train	SASS Train	POM Train	POM Train	POM Train	POM Train	POM Train	SASS Train
Anthracene/phenanthrene	0.0082	0.0114	0.0069	0.0745	0.1463	0.0618	0.1036	0.0104
Methyl-anthracenes/ -phenanthrenes	0.0027	0.0036	0.0083	0.0211	0.0510	0.0167	0.0513	0.0028
C$_2$-alkyl-anthracenes/ -phenanthrenes	0.0014[b]	0.0011	0.0014	0.0040	0.0070	0.0045	0.0094	0.0008
Cyclopenta-anthracenes/ -phenanthrenes	0.0014[b]	0.0004	0.0014	0.0032	0.0086	0.0030	0.0047	0.0002
Fluoranthene	0.0014[b]	0.0026	0.0016	0.0180	0.0316	0.0208	0.0188	0.0012
Pyrene	0.0014[b]	0.0026	0.0016	0.0156	0.0240	0.0169	0.0188	0.0013
Methyl-fluoranthenes/ -pyrenes	0.0014[b]	0.0023	0.0016	0.0128	0.0167	0.0103	0.0142	0.0016
Benzo[ghi]fluoranthene	—	0.0009	0.0014	0.0048	0.0067	0.0047	0.0067	0.0004
Cyclopenta[cd]pyrene	0.0014[b]	0.0010	0.0014	0.0048	0.0089	0.0051	0.0138	0.0005
Benzo[c]phenanthrene	—	0.0004	0.0013	0.0016	0.0023	0.0016	0.0044	0.0002
Benz[a]anthracene/ chrysene	0.0014[b]	0.0020	0.0014	0.0125	0.0138	0.0076	0.0371	0.0013
Methyl-benzanthracenes -benzphenanthrenes/ -chrysenes	0.0014[b]	0.0013	0.0016	0.0062	0.0104	0.0062	0.0048	0.0009
C$_2$-alkyl-benzanthracenes/ -benzophenanthrenes/ -chrysenes	—	0.0009	0.0014	0.0055	0.0044	0.0037	0.0047	0.0005
Benzofluoranthenes	0.0014[b]	0.0022	0.0016	0.0128	0.0159	0.0112	0.0141	0.0015
Benzopyrenes/perylene	0.0014[b]	0.0017	0.0014	0.0083	0.0116	0.0084	0.0094	0.0011
Methylcholanthrene	—	—	—	0.00007	—	—	—	—
Indeno[1,2,3-cd]pyrene								
Benzo[ghi]perylene	—	0.0013	0.0015	0.0045	0.0099	0.0043	0.0048	0.0011
Anthanthrene	—	—	—	—	—	—	—	—
Dibenzanthracenes/ -o-phenanthrenes	—	0.0003	0.00005	0.0007	0.0014	0.0010	0.00005	0.0002
Dibenzocarbazoles	—	—	—	—	—	—	—	—
Dibenzopyrenes	—	0.0007	0.0001	0.0011	0.0010	0.0007	0.00002	0.0005
Total[c] (= column sum)	0.0249	0.0365	0.0360	0.2121	0.3715	0.18851	0.3187	0.0265

[a] Reprinted from DeAngelis *et al.*[10] POM train used an XAD-2 resin and cooler (reduction to 21°C) to trap organic gases. SASS (source assessment sampling system) train used three cyclones, a filter for particle-size fractionation, an XAD-2 trap, and a trace inorganic impinger trap. Blanks indicate not detected.

[b] Compound was identified, but not quantified, because of the detection limits of the analytic method.

[c] The detection limit was taken as the emission factor for compounds that were identified, but not quantified.

TABLE 2-11

POM Emission Factors[a]

Compounds	Emission Factor, mg/kg[b]	
	0.12-m Logs, 0.82-kg/h Burn Rate	0.06-m Logs, 7.73-kg/h Burn Rate
Phenanthrene/anthracene	0.88	2.30
C_1-Phenanthrenes/anthracenes	0.42	0.18
C_2-Phenanthrenes/anthracenes	0.11	0.04
Cyclopentaphenanthrenes/anthracenes	ND	ND
Pyrenes	0.33	1.39
Fluoranthene	0.25	0.10
Benzo[a]fluorene	0.26	0.26
Unidentified POMs	0.70	1.31
C_1-Fluoranthenes/pyrenes	0.10	ND
Benzo[ghi]fluorene	0.04	0.10
9-Phenylanthracene	0.04	0.20
C_3-Phenanthrenes/anthracenes	0.20	0.03
Benzo[ghi]fluoranthene	ND	ND
C_2-Pyrenes	0.08	ND
Cyclopenta[cd]pyrene	ND	ND
Benzo[c]phenanthrene	ND	ND
Benz[a]anthracene/chrysene	0.44	1.35
Higher-molecular-weight POMs	0.25	1.26
Total	4.10	8.52

---

[a] Adapted from Hubble *et al.*[16]

[b] ND = not detected.

TABLE 2-12

Estimation of Annual Emission of POM Contributed by Various Sources[a]

Source	Annual Emission of Total POM	
	Metric tons	%
Residential heating:		
Wood-fired (total):[b]	3,837	34.8
Coal-fired	102	0.9
Oil-fired	7.4	0.1
Gas-fired	9.8	0.1
Open burning:		
Agriculture open burning	1,190	10.8
Prescribed burning	1,071	9.7
Forest wild fires	1,478	13.4
Coal-refuse fires	28.5	0.3
Land-clearing waste burning	171	1.6
Structural fires	86	0.8
Coke production	632	5.7
Mobile sources:		
Auto--gasoline	2,160.8	19.6
Auto--diesel	1.2	0.1
Trucks--diesel	103.3	0.9
Industrial boilers:		
Coal	69.0	6.3
Gas	2.1	0.1
Oil	1.3	0.1
Wood/bark	1.2	0.1
Bagasse	0.3	0.1

---

[a] Tabulated from Peters[36] for the early to middle 1970s.

[b] Primary heating, auxiliary heating, and fireplaces responsible for 1,383, 2,376, and 78 metric tons, respectively.

TABLE 2-13

Emission of PAHs in Wood Smoke[a]

PAH	Emission, mg/kg of wood burned				
	Fireplace	Baffled Stove		Nonbaffled Stove	
	Seasoned Oak	Seasoned Oak	Seasoned Pine	Seasoned Oak	Green Pine
Anthracene/ phenanthrene	8	75	146	62	103
Methyl(anthracenes/ phenanthrenes)	3	21	51	17	51
Fluoranthene	<1	18	32	21	19
Pyrene	<1	16	24	17	19
Methyl(fluoranthenes/ pyrenes)	<1	13	17	10	14
Benzo[ghi]-fluoranthene	--	5	7	5	5
Cyclopenta[cd]pyrene	<1	5	9	5	14
Benzo[c]phenanthrene	--	1	2	2	5
Benz[a]anthracene/ chrysene	<1	13	14	8	37
Benzofluoranthenes	<1	13	16	11	14
Benzopyrenes/perylene	<1	8	12	8	9
Benzo[ghi]perylene	--	1	1	1	0

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[a] Data from Peters.[36]

TABLE 2-14

Emission Factors for PAHs from Residential Combustion Sources as a Function of Thermal Efficiency[a]

Combustion Source	Approximate POM Emission Factor, pg/J (fuel input)	Average Thermal Efficiency, %	Approximate POM Emission Factor, pg/J (thermal output)
Wood-burning stove	17,000	55	31,000
Wood-burning fireplace	2,000	10	20,000
Automatic coal furnace	1,900	60	3,200
Oil furnace	4	70	5.7
Gas furnace	1	85	1.2

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[a] Data from Peters.[36]

TABLE 2-15

Polycyclic Aromatic Hydrocarbon Emission Summary by Incineration Source[a]

Type of Unit	Sampling Point	Group 1 Benzo[a]pyrene, µg/1,000 m³ [b]	Benzo[a]pyrene, µg/lb of refuse charged	Pyrene	Benzo[e]-pyrene	Perylene	Benzo[ghi]-perylene	Anthanthrene	Coronene	Group 2 Anthracene, µg/lb of refuse charged	Phenanthrene	Fluoranthene	Benz[a]-anthracene
Municipal:													
250-ton/d multiple chamber	Breeching (ahead of settling chamber)	19	0.075	8.0	0.34	—	—	—	0.26	—	—	9.8	0.37
50-ton/d multiple chamber	Breeching (ahead of scrubber)	2,700	6.1	52	12	—	26	—	15	—	18	4.6	—
	Stack (behind scrubber)	17	0.009	2.1	0.58	—	0.63	—	0.63	—	—	3.3	0.15
Commercial:													
5.3-ton/d single chamber	Stack	11,000	53	320	45	3.1	90	6.6	21	47	160	220	4.6
3-ton/d multiple chamber	Stack	52,000	260	4,300	260	60	870	79	210	86	59	3,900	290

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[a] Reprinted from Environmental Protection Agency. Review of Literature. Cited in Hangebrauck et al.[14] — = not detected in the sample.

[b] Micrograms per 1,000 m³ of flue gas at standard conditions (70°F, 1 atm).

TABLE 2-16

Emission of Polycyclic Aromatic Hydrocarbons[a]

Compound	Stack Gases		Residues		Water		
	mg/1,000 m	mg/d[b]	µg/kg	mg/d[c]	Input, µg/L	Output, µg/L	Output, mg/d[d]
Fluoranthene	0.58	274	58	1,360	0.08	0.62	15.5
Pyrene	1.58	745	49	1,150	0.08	0.54	13.5
Benz[a]anthracene + chrysene	0.72	340	171	4,010	0.03	0.64	16.0
Benzo[b]fluoranthene + benzo[k]fluoranthene + benzo[j]fluoranthene	0.32	151	292	6,850	0.03	0.14	3.5
Benzo[a]pyrene + benzo[e]pyrene	0.02	9	147	3,450	0.03	0.14	3.5
Perylene	0.18	85	82	1,920	0.02	0.13	3.3
Benzo[ghi]perylene	0.42	198	47	1,100	0.01	0.03	0.8
Indeno[1,2,3-cd]pyrene	0.18	85	10	230	0.01	0.01	0.3
Coronene	0.04	19	20	470	0.01	0.01	0.3

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[a] Reprinted with permission from Davies *et al.*;[8] copyright 1976 American Chemical Society.

[b] Assuming 12 h daily operation; levels measured at flow 655 m^3/min corrected to dry, 20°C, 760 mm Hg.

[c] Assuming 23% average moisture content and 30-metric-ton daily output.

[d] Assuming a discharge of 25,000 L/d from the quench trough.

TABLE 2-17

PAH Emission from a Secondary Lead Smelter Processing Batteries[a]

Compound	Emission Concentration,[b] ng/Nm^3			
	1A	1B	2A	2B
Anthracene/phenanthrene	600	740	770	940
Methylanthracenes	25	34	41	33
Fluoranthene	160	170	330	310
Pyrene	31	22	28	30
Methylpyrenes/fluoranthenes	2	2	3	2
Benzo[c]phenanthrene	10	11	17	13
Chrysene/benz[a]anthracene	25	23	28	25
Benzo[a]pyrene	1	1	1	1

---

[a] Data from Bennett *et al.*[2]

[b] N = corrected to 70°F and 1 atm. Sampling runs 1 and 2 were taken during separate visits to the smelter. Gas stream was split, and two simultaneous samples, A and B, were taken.

TABLE 2-18

PAHs from Burning of Pine Needles, by Fire Type[a]

PAH	Concentration, ng/g of fuel burned (dry weight basis)					
	Backing fires			Heading Fires		
	0.1 lb/ft	0.3 lb/ft	0.5 lb/ft	0.1 lb/ft	0.3 lb/ft	0.5 lb/ft
Anthracene/phenanthrene	12,181	2,189	584	2,525	5,542	6,768
Methylanthracene	9,400	1,147	449	1,057	4,965	7,611
Fluoranthene	14,563	2,140	687	733	974	1,051
Pyrene	20,407	3,102	1,084	1,121	979	1,133
Methylpyrene/fluoranthene	18,580	2,466	1,229	730	1,648	2,453
Benzo[c]phenanthrene	8,845	1,808	468	244	142	175
Chrysene/benz[a]anthracene	28,724	5,228	2,033	581	543	836
Methylchrysene	17,753	1,891	877	282	1,287	1,559
Benzofluoranthenes	12,835	1,216	818	164	129	241
Benzo[a]pyrene	3,454	555	238	38	40	97
Benzo[e]pyrene	5,836	1,172	680	61	78	152
Perylene	2,128	198	134	33	24	46
Methylbenzopyrenes	6,582	963	384	65	198	665
Indeno[1,2,3-cd]pyrene	4,282	655	169	—	—	—
Benzo[ghi]perylene	6,181	1,009	419	—	—	—
Total	171,750	25,735	10,249	7,632	16,549	22,787
Total suspended particulate matter (TSP)	21 lb/ton	9 lb/ton	5 lb/ton	20 lb/ton	73 lb/ton	118 lb/ton
Benzene-soluble organic substances	55%	50%	45%	44%	73%	75%

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[a] Reprinted with permission from C. K. McMahon and S. N. Tsoukalas. "Polynuclear aromatic hydrocarbons in forest fire smoke," pp. 61-73. In P. W. Jones and R. I. Freudenthal, Eds. Polynuclear Aromatic Hydrocarbons. Vol. 3. Carcinogenesis. New York: Raven Press, 1978. Moisture content for all fires ranged between 18% and 27%.

TABLE 2-19

Estimated Total Annual Benzo[a]pyrene Emission for 1975 and 1985

Estimate	Benzo[a]pyrene Emission, metric tons 1975	1985
Minimum	346	67
Intermediate	588	358
Maximum	1,676	885

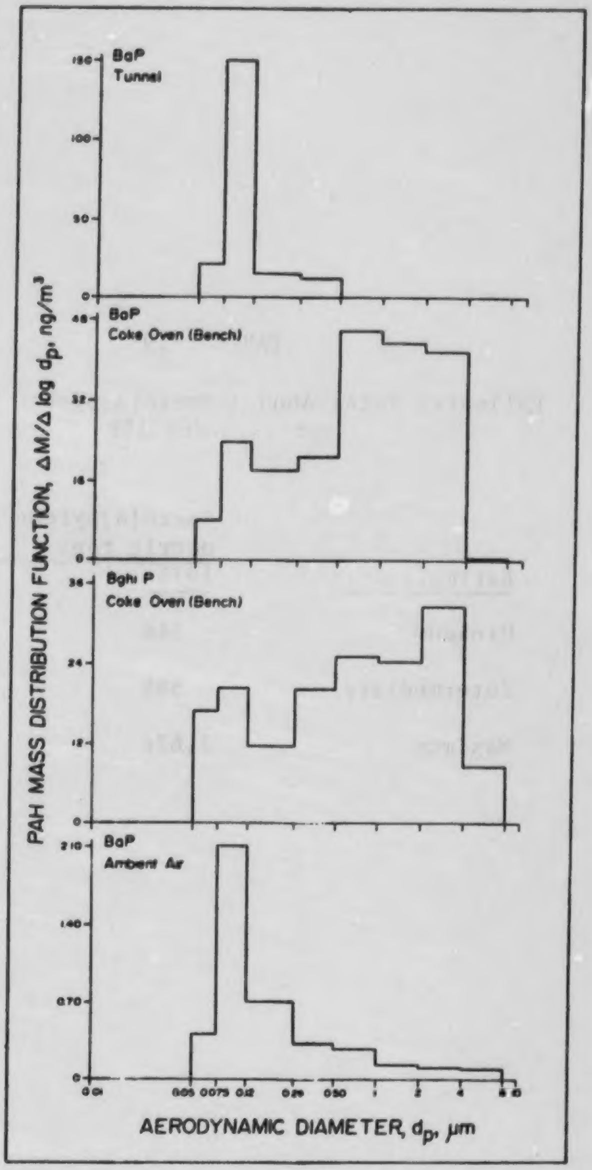

FIGURE 2-1. Distribution of BaP and BghiP as function of aerodynamic diameter. Total PAH concentrations, in ng/m^3: tunnel, 42.8 BaP; coke oven, 58.5 BaP and 44.2 BghiP; and ambient air, 1.01 BaP. Reprinted with permission from Miguel and Rubenich.[24]

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3

ATMOSPHERIC TRANSFORMATIONS OF POLYCYCLIC AROMATIC HYDROCARBONS

GENERAL CONSIDERATIONS: PERSISTENCE AND TRANSFORMATIONS OF PAHs

The atmospheric persistence of PAHs has received considerable attention in recent years and continues to be actively investigated. Two extreme situations can be envisioned. In the absence of any chemical interaction, the lifetime of PAHs adsorbed onto particles will depend solely on physical characteristics--the size of the carrier particle and scavenging processes, including wet and dry deposition. In addition, carrier-particle size is also critical with respect to the rate of deposition in (and clearance from) the human respiratory system and the rate of elution from the carrier particle by the lung tissue. Because submicrometer particles have atmospheric residence times of several days, experimental evidence on long-range atmospheric transport of PAHs and their distribution in sediments appears to support a hypothesis of negligible chemical transformation of PAHs in the atmosphere. Given the same carrier-particle residence time, even relatively slow chemical reactions could compete effectively with physical processes, with respect to PAH removal from the atmosphere. A substantial body of experimental evidence has been accumulated on chemical reactions between PAHs and pollutant gases under laboratory conditions, with reaction times as short as a few hours. The products of these reactions are in some cases much more potent mutagens than the parent PAHs, thus warranting concern about the implications of these chemical transformations with respect to human exposure.

The foregoing considerations suggest the format of this chapter. Pertinent information concerning the chemical and physical processes governing atmospheric persistence of PAHs is summarized first, followed by chemical reactions of PAHs, with an attempt to organize the somewhat conflicting published data according to reactant species and substrate, i.e., carrier particles and other substrates, including filters.

PAH FORMATION: CHEMISTRY AND PHYSICS

CHEMISTRY

The exact synthetic chemistry that produces PAHs in a fuel-rich flame is not well known, but PAHs can be produced from almost any fuel burned under oxygen-deficient conditions.[2] As an example of the PAH assemblage produced by combustion systems, Figure 3-1 (top) shows identified gas-chromatographic mass-spectrometry (GC/MS) peaks on PAHs produced by the combustion of kerosene.[59] Note that fluoranthene (peak 22) and pyrene (peak 25) are present in about equal abundances; that the abundance of phenanthrene (peak 14) far exceeds that of anthracene (peak 15), a less stable compound; and that benzo[a]pyrene (peak 39) is always found with its noncarcinogenic isomer benzo[e]pyrene (peak 38).

A particularly interesting group of compounds in combustion effluents are those with a vinylic bridge, such as acenaphthylene (peak 4) and cyclopenteno[cd]pyrene (peak 32). Peak 23, although not labeled, has been positively identified as acephenanthrylene,[54] which also has a vinylic bridge. We emphasize this structural feature because of its chemical reactivity (compared with that of the fully aromatic portions). This reactivity is important in considering the fate of PAHs in the atmosphere.

The PAHs shown in Figure 3-1 (top) are typical of those produced from the combustion of various fuels. The combustion of almost any fuel will produce the mixture of compounds shown. The relative abundances, however, can be substantially different, depending on the temperature of combustion. In fact, the relative abundances of the alkyl homologues of PAHs depend heavily on the temperature at which the fuel is burned. Although Figure 3-1 shows very modest amounts of alkyl homologues (see the region between peaks 25 and 30), other fuels burned under other conditions can show considerably greater abundances of alkyl PAHs.

PHYSICS

Adsorption

PAHs are formed in almost all combustion processes. As the effluent temperature decreases, PAHs initially present largely as vapors become adsorbed on condensing carriers, such as soot and fly ash.[38,67] It is generally accepted that the adsorption process is virtually completed at or near the point of emission into the ambient air, and that PAHs in ambient air are adsorbed on carrier particles. Studies of the distribution of selected PAHs between the gaseous and particulate phases in ambient air[6,7] have shown that, even though the smaller PAHs (e.g., with three and four aromatic rings) may have measurable gas-phase concentrations, these are, as a result of adsorption, lower by several orders of magnitude than those expected on the basis of the corresponding vapor pressures (see Table 3-1).

Particle Size Distribution

Once PAHs are adsorbed onto carrier particles, their size distribution in the atmosphere is governed by aerosol dynamics, including coagulation and condensation processes. Thus, carrier particles may evolve into substantially different "stable" size distributions. In many combustion processes, PAHs are emitted in the so-called nucleation mode, i.e., adsorbed on particles less than 0.1 μm in diameter. In diesel-engine exhaust, the carrier-particle distribution has mass median diameters of about 0.1-0.25 μm (National Research Council, unpublished manuscript). The contributions from various anthropogenic emission sources may have significant effects on the size distribution of airborne PAHs.

In early studies of the PAH size distribution in urban air, DeMaio and Corn[15] reported that most of the benzo[a]pyrene (BaP) was found to be associated with small particles (less than 2.3 μm in diameter). Kertész-Saringer and co-workers[47] reported that 50% of the BaP in Budapest air was found in particles smaller than 0.3 μm. Size distribution measurements were later extended to other PAHs, including benzo[k]fluoranthene,[1] 8-PAH and 2-PAH quinones,[72] 4-azaarenes, and 3-alkyl-substituted PAHs.[87] More recently, the application of new size-segregating sampling devices, such as the low-pressure impactor, has given more detailed information on the distribution of PAHs in the submicrometer range. Miguel and Friedlander[64] reported on the distribution of BaP and coronene in Pasadena, California, ambient air (Table 3-2). The largest concentration of both PAHs was found in particles with aerodynamic diameters between 0.075 and 0.12 μm.

The influence of particle size on human respiratory uptake has been the subject of a number of theoretical and experimental studies.[5,16,68] In the specific case of PAHs, it has been conclusively shown that the rate of uptake by lung membranes is much higher for PAHs adsorbed on physiologically inert carrier particles than for the same PAHs inhaled in the crystal state.[12,56] In addition, simultaneous measurements of carrier-particle and BaP clearance from the respiratory tract of mice for small (0.5-1.0 μm) and large (15-30 μm) carbon particles[12] showed that, even though smaller particles were cleared from the respiratory tract faster than larger ones, more BaP was eluted from small particles than from the large ones. These results are consistent with findings in tumorigenesis studies of Farrell and Davis,[18] in which the BaP-carbon combination of the smallest particles (0.5-1.0 μm) was the most carcinogenic, and underline the importance of PAH size distribution for human toxicity. However, most respiratory deposition-clearance studies have been limited to two sizes (i.e., about 1 μm and over 10 μm), and no information is available on the effect of carrier-particle size on PAH retention in the range of interest, i.e., less than 0.25 μm. Such size resolution would provide valuable information not only on PAH retention from ambient particles, but also on the relative contribution of various emission sources to PAH uptake.

PHYSICAL REMOVAL PROCESSES FOR ATMOSPHERIC PAHs

Once PAHs are released from the combustion system and adsorbed on soot or fly ash, they are exposed to potential atmospheric degradation. In the absence of major photodecomposition or other chemical transformations, PAHs would be removed from the atmosphere by dry and wet deposition.[73] Dry deposition involves sedimentation, turbulence-induced collision with surface electrostatic deposition, and inertial impaction. Although settling velocities have apparently not been determined for PAHs, it is generally accepted that they are controlled by those of the carrier particle.

Carrier-particle settling velocities can be estimated from Stokes's law, i.e., assuming that the settling velocity is proportional to the square of the particle diameter, to a term that includes the particle-to-fluid (air) density ratio, and to the reciprocal of the fluid viscosity. Thus, for a 1-μm particle with a density of about 2 g/cm^3 in air at 20^oC, the settling velocity is about 6×10^{-5} m/s,[90] in agreement with experimentally determined velocities of about 10×10^{-5} m/s for 1-μm particles.[80] For such a particle suspended in air at a height of 20 m and with an average wind speed of 4 m/s (about 9 mph), it would take 4 d to settle to the surface. Assuming a constant wind speed of 4 m/s and constant wind direction over the 4-d period, this atmospheric residence time is equivalent to atmospheric transport over a distance of 1,400 km. Experimental evidence of such regional- and subcontinental-scale transport of PAHs in the atmosphere is discussed below.

A simple way in which to note the relative degradation susceptibility of the various PAHs is to compare the GC/MS data on PAHs coming from a combustion system (see Figure 3-1, top) with the PAH profile of atmospheric particles (Figure 3-1, middle). PAHs without vinylic bridges are still prevalent, the ratio of fluoranthene to pyrene is still about 1:1, and the ratio of phenanthrene to anthracene is about 10:1. Compounds with vinylic bridges (acenaphthylene, peak 14; acephenanthrylene, peak 23; and cyclopenteno[cd]pyrene, peak 32) have completely vanished from the PAH mixture found in the atmosphere. The increased chemical reactivity of the relatively localized double bond found in these compounds apparently makes them susceptible to photolytic oxidation.

Assuming that most PAHs are stable in the atmosphere, what happens to these compounds after they are released from combustion systems throughout the world? Two types of data address this question: data on PAHs in marine and lacustrine sediments, presumably the ultimate environmental sinks of atmospheric PAHs; and data on PAHs in air sampled at remote locations.

PAHs IN MARINE AND LACUSTRINE SEDIMENTS

Many workers have observed significant concentrations of PAHs in aquatic sediments. For example, Figure 3-1 (bottom) shows a GC/MS analysis of PAHs in the sediment of the Charles River. In comparing the bottom and the middle of Figure 3-1, one sees considerable resemblance. The ratios of the major groups of compounds are the same; the PAHs with vinylic bridges are missing, as they were in the atmosphere; and the alkyl homologues are about as abundant as one might expect. Similar data have been obtained, but in a more quantitative fashion, on over 50 sediment samples from around the world.[35] These data indicate that PAHs are ubiquitous and that they are found in almost all samples both near and remote from urban areas. The PAH pattern in all these samples, even the most remote, is similar to that shown in Figure 3-1 (bottom).

Even though the relative distribution remains constant, the total amount of PAHs decreases dramatically with distance from urban centers. Figure 3-2 shows a plot of the total PAH abundance in five marine-sediment samples taken from Massachusetts Bay as a function of distance from Boston.[90] There is a decrease by 3 orders of magnitude in the total abundance of PAHs within 100 km of Boston. At that point, the total PAH concentration is about 100 ppb; remarkably, that is what is seen in almost all other samples from areas remote from urban centers.

On the basis of these and other measurements of PAH concentrations, the following scenario is suggested for the transport of PAHs. The various fuels that are burned in metropolitan areas produce airborne particulate matter (soot and fly ash) on which PAHs are adsorbed. These particles are transported by the prevailing wind for distances that depend heavily on particle diameter. The long-range airborne transport of small particles may account for the presence of PAHs in deep-ocean sediments.

Larger airborne particles will settle back onto the urban area; rain then washes them from streets and buildings. The PAHs in this urban runoff eventually accumulate in local sinks. These highly contaminated sediments could be slowly transported by resuspension and currents to seaward locations, where the sediments accumulate in basins or in the deep ocean. The rapid decrease in PAH concentration to 100 ppb within 100 km of Boston (see Figure 3-2) indicates that this transport mode is a rather short-range effect.

The stability of PAHs is also apparent when one examines sediment samples taken in such a way as to preserve the historical record. This can be done by carefully coring sediments, segmenting the core into 2-4-cm sections, and analyzing each section for PAHs quantitatively. An example of such data is shown in Figure 3-3; this represents a core from the Pettaquamscutt River in Rhode Island, an anoxic basin.[35] The total PAH concentrations range from 14,000 ppb near the sediment surface to less than 120 ppb at the core bottom. Despite the range of concentrations, the relative distribution of the PAHs (excluding the natural products retene and perylene) is indicative of combustion. For example, the ratio of the $C_{16}H_{10}$ isomers (nonalkylated) to their monoalkyl homologues ($C_{17}H_{12}$) is 3.0 ± 0.4:1. In no case does this ratio become less than unity, which would be expected if the source were direct fossil-fuel contamination. The ratio of the $C_{16}H_{10}$ isomers to the $C_{18}H_{12}$ isomers is 2.7 ± 0.3:1, and the ratio of the $C_{14}H_{10}$ isomers to the $C_{20}H_{12}$ isomers is 0.46 ± 0.08:1. These ratios are consistent throughout the core and are indicative of combustion sources.[55] Combustion seems to have been the source of the PAHs in all sections of the core.

With a reported deposition rate of 3 mm/yr, total PAHs (excluding retene and perylene) in the Pettaquamscutt core were plotted against year of deposition (see Figure 3-3). For comparison, the BaP data reported by Grimmer and Böhnke[27] for a core from the Grosser Ploner Sea are also plotted in Figure 3-3. The similarity between these two core profiles is remarkable. Both show rapid increases in PAH concentrations beginning around 1900. The increases could be due to the heavy industrialization that occurred at the turn of the century and the combustion associated with it.

A slight decrease in total PAHs around 1930 is present in both cores (see Figure 3-3). It is intriguing to speculate that this reflects an event that occurred in both Europe and New England at this time. The Depression could be such an event. During the Depression, total U.S. energy consumption decreased from 25 x 10^{15} BTU (in 1929) to 18 x 10^{15} BTU (in 1932) before resuming its increasing trend.[36]

The Pettaquamscutt data are from a core deep enough to allow the assessment of the PAH burden before 1900. The PAH concentrations are low and constant (about 200 ppb) for the 50 yr before the turn of the century. That may be indicative of PAHs from natural combustion processes, such as forest fires. Contributions from natural processes appear to be insignificant in areas or periods of high anthropogenic activity.

The decrease in PAHs after 1950 is interesting. It may reflect the change from coal to oil and natural gas as home heating fuels that occurred in the 1950s. During the period 1944-1961, the use of coal in the United States decreased by 40%, and the use of oil and gas increased by 20%.[36] Combustion of coal usually produces more PAHs than oil and gas, so the change in fuel would result in a decrease in PAH production during the same period. A return to coal as a major energy source without stringent emission controls might therefore have an important effect on man's input of PAHs into the atmosphere and the sedimentary environment.

In an effort to measure the deposition rates for PAHs from the atmosphere in both remote and urban locales, PAH concentrations in sediment cores from water bodies in several areas in the northeastern United States have been determined, and the corresponding atmospheric PAH fluxes to these sites have been calculated. In assessing flux information (rather than concentrations), many of the differences between sites are taken into account, thereby allowing useful comparisons. PAH fluxes calculated for lakes on islands and for remote high-altitude lakes were particularly interesting, in that these sites should reflect most accurately the atmospheric deposition of these combustion-derived pollutants. This background flux could then be compared with PAH inputs found nearer to urban centers, thereby showing the relative importance of long-range airborne vs. short-range runoff delivery of PAHs.

With the observed PAH concentrations and information on the sedimentation rates and in situ dry densities, the fluxes of individual PAHs to five remote and three urban sites were calculated.[32] Table 3-3 (top) shows the results of these flux calculations for core subsections reflecting PAH deposition in remote sites at present, in the interval including 1950, and at the turn of the century (1900). The first point to notice is that the average fluxes for most individual PAHs (except anthracene) to remote northeastern U.S. sites are 0.8-3 ng/cm^2 at present.

Where core subsampling resolution permits, it can be seen that atmospheric PAH fluxes approximately 30 yr ago were 2-3 times greater than and those around 1900 were one-tenth to one-fifth of the present flux rates. This historical PAH record clearly shows that man's activities over the last century resulted in an influx of PAHs to the environment and that coal-derived energy was a much greater source of polluting PAHs than energy derived from oil and gas.

For comparison, similar flux estimates for three sites much closer to urban centers were calculated. The results are shown in Table 3-3. These locations all have much greater PAH fluxes than the remote locations. As suggested above, such locations probably receive most of their PAH contamination via water runoff from the watershed. This source of PAHs in sediment overwhelms the background atmospheric deposition rates seen at remote sites.

In summary, several things are now apparent about the physical transport of PAHs from source to depot:

o PAHs (except retene and perylene) in continental aquatic sediments originate largely in anthropogenic combustion.

o The watershed runoff resuspension mechanism is of short range (about 100 km) and delivers a near-shore flux of about 35 ng/cm^2 per yr for an individual PAH.

o The airborne transport mechanism is of long range and delivers a flux of about 1 ng/cm^2 per yr for an individual PAH.

o Anthropogenic sources of PAHs were first observed in sediments at concentrations significantly higher than natural background in around 1900, and the maximal deposition was in about 1950.

We need more information about the mechanisms of PAH transport to remote sediments. For example, what fraction of the PAH flux is delivered by aquatic transport mechanisms and what fraction by atmospheric fallout? How much, if any, is lost to the water column?

PAHs IN AIR SAMPLED AT REMOTE LOCATIONS

Experimental evidence of long-range transport of PAHs has been presented by Lunde, Bjorseth, and co-workers.[6,61,62] They analyzed the PAH content of particulate samples collected in Norway with respect to air trajectory. As seen in Table 3-4, PAH concentrations in air masses originating in industrialized areas in western Europe were 20 times higher than those measured in air masses originating in Norway and were as high as those typically measured in urban and industrial areas. These results support the concept, at least for the 20 PAHs listed in Table 3-4 and collected during the winter (i.e., low temperature and low light intensity, resulting in little, if any, photochemical activity), of atmospheric transport of PAHs over long distances from anthropogenic sources.

CHEMICAL REMOVAL PROCESSES FOR ATMOSPHERIC PAHs

Chemical reactions of PAHs in the atmosphere have received steady attention for about 30 yr, as have their implications for human health. In their classic work demonstrating the presence of BaP and other PAHs in the Los Angeles atmosphere and the carcinogenicity of atmospheric organic particulate matter in mice, Kotin et al.[52] investigated the interactions between BaP deposited on filters and pollutant gases, including nitrogen dioxide (NO_2) and ozone (O_3). In this early study of the currently much investigated interactions between PAHs and oxides of nitrogen (NO_x) and the health implications of nitro-PAH compounds, Kotin et al. reported a 60% loss of BaP deposited on filter paper when it was exposed to NO_2. Later research has focused on BaP and a number of other PAHs; on photolysis and photooxidation, as well as on thermal reactions of PAHs with O_3, NO_x, and sulfur dioxide (SO_2); and on the influence of the physical and chemical nature of the substrate on the reactivity of adsorbed PAHs. The corresponding literature is somewhat conflicting, owing in part to the large number of characteristics that influence these complex and still only partially understood heterogeneous reactions. Thus, it is not surprising to note, even in the recent literature, statements to the effect that PAHs are not chemically reactive and are removed from the atmosphere by rain and sedimentation (e.g., Fishbein[20]). As discussed below, chemical reactions--including photooxidation, reactions with SO_2 and NO_x, and reactions with O_3 and other oxidants--may, in fact, constitute major pathways for removal of PAHs from the atmosphere. This discussion focuses on studies of the reactions of PAHs deposited or adsorbed on a variety of substrates (e.g., soot, silica gel, alumina, and glass-fiber filters). The corresponding literature concerning PAH chemistry in the bulk liquid phase (e.g., National Research Council[66]) is not included here, except for a few studies directly relevant to the chemistry of adsorbed PAHs.

REACTION OF PAHs WITH OZONE

Kotin et al.[52] first reported on the reaction of pure BaP deposited on a filter and exposed to various pollutants and mixtures of pollutants, including O_3, NO_2, and O_3 plus NO_2. More recently, Lane and Katz,[57] Pitts et al.,[74,75] and Katz et al.[45] have reported on the chemical half-lives of PAHs exposed to O_3 and on the nature and mutagenic activity of the products.

In experiments conducted with BaP, benzo[b]fluoranthene (BbF), and benzo[k]fluoranthene (BkF) exposed to O_3 (at 0.19-2.28 ppm) in air with and without irradiation, Lane and Katz[57] reported half-lives of about 40 min for BaP exposed to O_3 at 0.19 ppm in the dark. For the three PAHs studied, half-lives decreased with increasing O_3 concentration and were further reduced by irradiation with quartzline lamps (Table 3-5). Substantial differences in reactivity were observed, with BbF and BkF being some 10 times more resistant than BaP to ozonolysis, both in the dark and under irradiation. Katz et al.[45] extended this study to nine PAHs deposited on cellulose thin-layer chromotography (TLC) plates and exposed to O_3 at 0.2 ppm in the dark, simulated sunlight, and both. The corresponding results (Table 3-5) show significant ozonolysis of some PAHs in the dark, with half-lives ranging from about 0.6 h for BaP and 1.2 h for anthracene to 7.6 h for BeP. Pyrene (half-life, about 16 h), BkF (35 h), and BbF (53 h) were more resistant to dark ozonolysis. For seven of the nine PAHs studied, half-lives were further reduced by irradiation. Pitts et al.[74,75] also determined a half-life of about 1 h for BaP deposited on a glass-fiber filter and exposed to O_3 at about 0.2 ppm. The results, including the direct comparison between adsorbed and liquid-phase data reported by Katz et al.,[45] clearly demonstrate that the reactivity of PAHs with O_3 is much greater for PAHs deposited on solid substrates than for PAHs in the bulk liquid phase.

Products of the reactions between BaP and O_3 have been analyzed. Katz et al.[45] identified the 1,6-, 3,6-, and 6,12-diones as major products and noted that all three BaP diones had been identified by Pierce and Katz[71] in ambient Toronto air. Pitts et al.[74] also reported the epoxide BaP 3,4-oxide as a reaction product. Van Vaeck et al.[86] identified a variety of reaction pathways and tentative structures of oxygenated reaction products of the gas-phase ozonolysis of BaP as shown in Figure 3-4. With respect to health implications, Katz et al.[45] stated that the BaP diones are direct-acting mutagens, but Pitts et al.[75] found these products inactive in the Salmonella/microsome assay. BaP 4,5-oxide, a DNA-binding metabolite of BaP, is a strong, direct-acting mutagen.[74]

REACTIONS OF PAHs WITH OXIDES OF NITROGEN

Kotin et al.[52] reported substantial (60%) loss of BaP deposited on a filter and exposed to NO_2. The high activity of nitro derivatives of PAHs--many of which are potent, direct-acting mutagens[63,85]--has prompted renewed interest in the possible formation of these compounds in the atmosphere by reaction of adsorbed PAHs with coemitted NO_x. Recent studies discussed here include those of Jäger,[41] Gundel et al.,[33] Pitts et al.,[75] Hughes et al.,[37] Jäger and Hanus,[42] Butler and Crossley,[7] and Tokiwa et al.[84] With the exception of Butler and Crossley,[7] who used a mixture of nitric oxide (NO) and NO_2, all studies have focused on NO_2. Hughes et al.[37] reported no reaction between NO and PAHs adsorbed on coal fly ash, alumina, and silica gel. A list of the 14 PAHs studied to date is given in Table 3-6. The molecular structures of corresponding nitro-PAH products are shown in Figure 3-5.

In all product studies cited above, exposure of adsorbed PAHs to NO_2 at parts-per-million concentrations resulted in the formation of nitro-PAH derivatives. These are also listed in Table 3-6 and include mononitro as well as dinitro derivatives, the latter identified as nitration products of BaP[42] and pyrene.[37] In the study of Jäger and Hanus,[42] dinitro-BaP was readily produced under conditions relevant to air pollution, i.e., by exposure of BaP adsorbed on fly ash to NO_2 at 1.33 ppm for 4 h at 20°C. Tokiwa et al.[85] have reported extremely high mutagenic activity for dinitro-PAHs as direct mutagens in the Salmonella/microsome test (e.g., 192 x 10^3 revertants/nmol for 1,6-dinitropyrene with strain TA 98 without metabolic activation). Mononitro-PAHs, although not as potent mutagens as their dinitro homologues, also exhibit substantial activity as direct mutagens.[63,77,85] Two nitro-PAHs, 1-nitropyrene and 3-nitrofluoranthene, are carcinogenic in rats.[69]

The effect of substrate on the nitration of adsorbed PAHs has recently been investigated. Hughes et al.[37] compared silica gel, alumina, and coal fly ash and noted that the extent of nitration of pyrene depends on the acidity of the substrate. Jäger and Hanus[42] discussed the effect of PAH structure, substrate chemical and physical characteristics, NO_2 concentration, temperature, and exposure time on the yields of nitro products of pyrene and BaP. For both PAHs, the yields of nitro products were substrate-dependent according to the sequence silica gel > fly ash > alumina >carbon (soot), with silica gel-to-carbon yield ratios as large as a factor of 280 for nitropyrene (Table 3-7). As expected, nitration yields increased as a function of NO_2 concentration and exposure time, but not necessarily in a straightforward manner, owing to such complex factors as the adsorption-desorption behavior of NO_2 on the substrate.

In view of the complex heterogeneous interactions involved, it is not surprising to note large differences in the nitro-PAH yields reported by several investigators. Tokiwa et al.[85] prepared nitro derivatives by exposure of pyrene, phenanthrene, fluorene, chrysene, and fluoranthene deposited on Toyo #2 paper filters to NO_2 for 24 h at 30°C in the dark. Large yields were obtained with NO_2 at 10 ppm, but yields of only a few percent were obtained at 1 ppm. These low yields are consistent with those reported by Jäger and Hanus[42] for BaP and pyrene exposure to NO_2 at 1.33 ppm for 4 h at 20°C with carbon as substrate. In contrast, Pitts et al.[75] reported 40% conversion of BaP deposited on glass-fiber filters and exposed to NO_2 at 1 ppm for 8 h at ambient temperature, and a yield of 18% after exposure to NO_2 at 0.25 ppm under the same conditions. The higher yields obtained on glass-fiber filters may be due to a greater catalytic effect of the glass-fiber filter than of soot (carbon) substrates.

In the same way, reported PAH half-lives due to reaction with NO_2 vary considerably with experimental conditions. From the above results, one can derive a half-life of 10 h for BaP in the study of Pitts et al.,[75] as opposed to half-lives of several days (or weeks) for several PAHs, including BaP, as investigated by Tokiwa et al.[84] and Jäger and Hanus.[42] Butler and Crossley[7] recently determined half-lives for 10 PAHs adsorbed on carbon (soot from a burner) and exposed to NO_2 at 10 ppm for up to 50 d. Their results, listed in Table 3-6, indicate PAH half-lives ranging from 4-7 d for the more reactive PAHs (anthanthrene, BaP, and benzo[ghi] perylene) to about a month for the least reactive compounds (phenanthrene, fluoranthene, coronene, and chrysene). These half-lives are consistent with those derived from the work of Jäger and Hanus[42] and Tokiwa et al.[84] In view of the substrate used (combustion-generated soot), the results of Butler and Crossley[7] and Jäger and Hanus[42] are probably applicable to heterogeneous nitration of PAHs in the atmosphere.

Given the high mutagenic potency of nitro-PAHs, it appears appropriate to speculate on the fate of these compounds in ambient air. Four nitro-PAHs have been reported in urban particulate matter--6-nitro-BaP,[41] 3-nitrofluoranthene,[41,85] 1-nitropyrene, and 5-nitroacenaphthene[85]--and indirect evidence of the presence of nitro-PAHs in Wayne County, Michigan, air has been presented on the basis of mutagenicity assays conducted with nitroreductase-deficient strains.[48,89] Photolysis of nitro-PAHs, such as 9-nitroanthracene, yields the corresponding diones (e.g., 9,10-anthraquinone), both in solution[9] and on silica gel.[75] On exposure of pyrene to NO_2, Jäger and Hanus[42] noted the appearance of new products after 4 h, and the nitropyrene yield decreased substantially. However, the retention times of these as yet unidentified compounds were different from those of the pyrene diones. The atmospheric relevance of these and other pathways should be investigated further.

REACTIONS OF PAHs WITH SULFUR DIOXIDE

Jäger and Rakovic[43] have reported the formation of sulfonic acids and other sulfur-containing compounds on exposure of BaP and pyrene (adsorbed on fly ash and on alumina) to SO_2 at a high concentration (10% in air). These sulfonic acids are also easily prepared in the liquid phase by reaction of PAHs with sulfuric acid at room temperature.[88] Tebbens et al.[82] observed significant consumption of BaP adsorbed on soot from a propane burner and exposed to SO_2 at 50-80 ppm for 4 h, both in the dark and under irradiation.

At lower SO_2 concentrations more relevant to ambient pollution, PAHs do not appear to react readily with SO_2. Hughes et al.[37] observed no reaction between SO_2 at parts-per-million concentrations and BaP or pyrene adsorbed on silica gel, alumina, and coal fly ash. Butler and Crossley[7] exposed 20 PAHs adsorbed on carbon (soot) to SO_2 at 5 ppm in air for up to 100 d. The matrix air contained water vapor, and thus some sulfuric acid was presumably present. Within the stated analytic precision, no significant reaction was observed for phenanthrene, coronene, fluoranthene, chrysene, BaP, pyrene, benz[a]anthracene, benzo[ghi]perylene, and anthanthrene. Because the 20 PAHs studied include both highly reactive and essentially inert compounds with respect to reaction with, for example, O_3 and NO_2, the conclusions of Butler and Crossley[7] as to the absence of significant reaction with SO_2 can probably be extended to many of the PAHs present in polluted air. However, SO_2 and sulfuric acid may play a role as catalysts for other PAH reactions, including nitration, and this possible catalytic role should be investigated.

REACTIONS OF PAHs WITH OTHER OXIDIZING SPECIES

Reactions of adsorbed PAHs with atmospheric pollutants other than O_3, SO_2, and NO_2 have received very little attention. Pitts et al.[75] exposed BaP deposited on a glass filter to peroxyacetylnitrate

[$CH_3CO(OO)NO_2$, or PAN] at about 1 ppm for 16 h and observed the formation of BaP diones and other oxidation products. Ambient concentrations of PAN in the Los Angeles atmosphere often reach 30 ppb during episodes of photochemical smog,[29] so PAN may contribute, with O_3, to the oxidative degradation of PAHs in photochemically polluted air. Reactions of PAHs with free radicals, including the hydroxyl (OH) and hydroperoxyl (HO_2) radicals (well documented in the bulk liquid phase), have not been studied in the context of atmospheric pollution. On the basis of studies conducted with aromatic compounds, such as toluene, the OH photooxidation products in the presence of NO_x include particulate-phase hydroxynitrotoluene and dihydroxynitrotoluene as major products.[31] It is possible that atmospheric oxidation of PAHs initiated by reaction with the OH radical results in the formation of nitro, hydroxy, and hydroxynitro derivatives.

PHOTOCHEMICAL REACTIONS OF PAHs

The mechanisms involved in photochemical reactions of PAHs with singlet oxygen in the bulk-liquid phase have received considerable attention and have been the object of several comprehensive reviews.[24,66] As is illustrated in Figure 3-6 for anthracene, products of these reactions include the PAH diones, formed either directly or by further reaction of primary endoperoxide products, as well as other oxygenated compounds.

Photomodifications of BaP and other PAHs in the adsorbed state have received significant attention with respect to both product distribution and influence of substrate. Product studies are in good agreement, and the chemical distribution of PAH phototransformations in the adsorbed state closely resembles that obtained in the bulk-liquid phase. However, reactivity reportedly varies widely as a function of substrate, and that makes it difficult to extrapolate laboratory studies to the ambient atmosphere.

Falk, Markul, and Kotin[17] first reported on the photodecomposition of 10 PAHs, including BaP, deposited on Whatman #1 paper filters and exposed to air in the dark, to air under irradiation, and to synthetic smog prepared by the reaction of O_3 with gasoline. Their experiments were conducted with pure PAHs, as well as with PAHs adsorbed on gasoline-engine exhaust soot. On exposure of light in air, all PAHs adsorbed on soot were more resistant to photodecomposition than the same compounds in the pure form. Tebbens *et al.*[81,82] investigated phototransformations of BaP, perylene, pyrene, and fluoranthene adsorbed on soot or deposited on paper, acetylated, and glass-fiber filters. Losses of BaP of up to 40% were observed on irradiation for some 45 min in air; the major reaction products were the three BaP diones and a carboxylic acid derivative. Thomas *et al.*[83] reported similar results for BaP.

Phototransformations of BaP and other PAHs have also been observed on a variety of substrates, including alumina,[39,51] silica gel,[39,40,51] cellulose,[39,45] acetylated cellulose,[39] soil,[19] carbon microneedles,[3] atmospheric particulate matter,[21] and coal fly ash.[50] A summary of the products of heterogeneous photooxidation of BaP on various substrates is given in Figure 3-7.

For comparison, Table 3-8 lists half-lives and percent losses determined for a number of PAHs deposited on cellulose TLC plates[45] and on Whatman #1 paper[17] and adsorbed on soot[17] and on fly ash.[50] Although the four sets of data are directly comparable for only two compounds, perylene and BaP, the effect of substrate on PAH phototransformations is evident. PAHs deposited in the pure form on cellulose TLC plates exhibit short half-lives, from 23 min for anthracene to about 20 h for BaP.[45] On Whatman paper, pure PAHs appear more resistant to photooxidation; e.g., the half-life for perylene is about 2 d, compared with only 4 h on cellulose TLC plates. In the adsorbed state, PAHs appear to be much more resistant to photooxidation, with losses of only 10% on soot after 48 h of irradiation.[17] On fly ash, only modest photodecomposition rates (up to 20%) are observed, in striking contrast with rapid photooxidation in the liquid phase and on silica gel.[50] If one neglects in a first approximation the important differences in experimental conditions, it appears from the data listed in Table 3-8 that PAHs adsorbed on atmospheric particles may be somewhat resistant to photooxidation, with half-lives ranging from several days to several weeks, depending on the reactivity of each compound.

Korfmacher et al.[50] have discussed the possible physical and chemical factors involved in the resistance of PAHs to photooxidation when adsorbed on fly ash. Resistance to photooxidation on soot, although even more relevant to urban pollution, where submicrometer particles contain a substantial fraction of carbonaceous material,[30] has not been fully investigated. In addition, specific PAH-substrate interactions have to be considered. For example, Korfmacher et al.[49] and Kotin et al.[53] observed rapid decomposition in the dark of some PAHs adsorbed on fly ash and on soot.

Until more data become available, caution must be exercised in extrapolating laboratory results to PAH photooxidation in the atmosphere.

INTERACTIONS OF DEPOSITED PAHs WITH AMBIENT POLLUTANTS

It is somewhat surprising, in view of the critical need to obtain overall PAH chemical deposition rates over a range of ambient conditions, that only a few studies have investigated interactions of PAHs with ambient polluted air. Pitts et al.[75] exposed pure BaP deposited on a glass filter to particle-free ambient Riverside, California, air for 3 d. BaP was partially oxidized under these conditions, yielding BaP diones and a variety of oxygenated (but not nitro) derivatives. In contrast, Fox and Olive[21] found only trace amounts of anthraquinone from anthracene (a reactive PAH in the studies discussed above) adsorbed on ambient particulate matter (suburban location near Austin, Texas) and exposed to atmospheric gases for 4 d in the dark. Comparison of the results of these two studies suggests that, as noted for photooxidation, PAHs appear more resistant to degradation in the adsorbed state than in the pure form. Peters and Seifert[70] exposed glass-fiber filters impregnated with ^{14}C-labeled BaP to ambient air in Berlin, Germany, and noted substantial losses of BaP, typically 75% over 24 h. Simultaneous determination of ^{14}C activity (only 10% loss in 24 h) established that BaP losses were due to chemical reaction, rather than to BaP evaporation from the filter. In addition, a relationship was noted between BaP reaction rates and ambient O_3 concentrations.

A recent investigation of PAH concentrations in the plume of a coal-fired power plant as a function of distance from the stack has been reported by Kalkwarf and Garcia.[44] Fluoranthene, BaP, pyrene, and BeP in the plume were found to be 50% reacted 3, 6, 8, and 12 km from the stack, respectively (with correction for plume dilution and dispersion). The loss of the PAHs was attributed to their reaction with coemitted NO_2 and SO_2.

PAHs IN AMBIENT AIR

Source identification is a key problem in the development of a pollution abatement or control program. In 1973, Friedlander[22] described a technique to identify the sources of air pollutants in emission inventories for particulate matter. Many chemical elements--such as sodium, chlorine, silicon, and aluminum--are found in natural background aerosols of the atmospheres of urban and industrial basins, such as Los Angeles. These are differentiated from other chemical tracers--such as lead, vanadium, zinc, and barium--which are attributable to human activities (see Figure 3-8). Thus, if some of the major sources are known in a given area, the source contributors to the atmosphere can be identified and calculated by measuring the elemental concentrations at a given point and fitting the data into a mathematical model. One of the major problems in using this technique has been the need for instrumentation for real-time measurement of the tracer elements. The use of trace metals for identification of sources of particles was examined by Moyers *et al*. in 1977.[65] With these tracers, several sources of particulate species in desert, rural, and urban atmospheres could be determined.

In 1979, Daisey *et al*.[14] described three methods for source identification for the PAHs in the complex mix of the atmosphere. Although the evaluations of these methods are in the early stages, it was found that statistical modeling does not depend on source emission data, if the ambient-air measurement data base is large. In 1981, Daisey and Kneip[13] reported that it was possible to use multivariate regression models of ambient-air data for apportioning the contributions of emission sources to airborne particulate organic matter. The contributing sources of respirable particles were determined by analysis of the ambient-air measurement data taken in New York City: 19% were from automobiles and related sources, 40% were from oil-burning, and 15% were soil-like particles. Although this study using tracer chemicals had good results, the methods should be validated for predictive use by testing in other locations.

A comprehensive discussion and critique of environmental sampling and analytic methods used for polycyclic organic matter are in the EPA report.[79] Lee et al.,[58] in a book on the analytic chemistry of PAHs, discussed sampling of mobile and stationary sources, ambient air, water, food, soils, and the aquatic environment. The cleanup and separation processes for the various collection media include solvent partitioning for analysis by column, paper, thin-layer, gas, and high-pressure liquid chromatography. The percentages of recovery with the analytic methods for the various PAHs were described by Lee et al., but are not discussed here.

In 1967, Hangebrauck et al.,[34] in a review of known sources of PAHs, gave the results of a survey made by the National Center for Air Pollution Control to screen the processes likely to produce emission in urban air. Although the survey was not intended to establish statistically the average emission from the sources, the data from it do characterize and classify the rate of emission of several PAHs from four major source categories: heat generation, refuse-burning, industrial processes, and motor vehicles. The 1972 NRC report Particulate Polycyclic Organic Matter[66] estimated that heat and power generation produced BaP at 500 metric tons/yr; refuse-burning, at 600 tons/yr; coke production, at 200 tons/yr; and motor vehicles, at 20 tons/yr. BaP has often been used as a surrogate in estimating source contributions of complex mixtures of PAHs.

Surrogate chemicals have been used commonly in monitoring environmental quality, for various reasons--e.g., analytic methods are often available only for the surrogate, and it costs less to monitor only one chemical. However, a PAH surrogate may not be useful unless studies have been conducted to characterize the PAH profile and percentage relationships for each type of environment. For example, in 1979, Bjorseth et al.[6] showed that the relative distribution of PAHs is not the same in all environments. Figure 3-9 shows comparisons of the percentages of PAHs found in the particulate matter from an aluminum plant and a Soderberg paste plant. He recommended that a parent PAH profile (PPP) be established before a surrogate compound was chosen. In 1981, Gammage and Bjorseth[23] stated that there are no established techniques for real-time monitoring of selected PAHs and that BaP is not a universally accepted proxy or surrogate for PAHs. It is known that the numerous PAHs found in the outdoor air can be radically different, qualitatively and quantitatively, from those in the workplace environment and that monitoring one compound as a surrogate for others is unreliable. The recommendation was made again that a PPP be determined before a proxy or surrogate compound was chosen.

Considerable information is available to show the profile of the various PAHs from various sources. Comparison of the rate of PAH emission between different categories is complicated, owing to the different units used to measure them: μg/BTU, μg/barrel of oil, μg/g of particles, μg/lb of material processed, μg/m^3, μg/mi. Qualitative comparisons of PAH emission sources do appear feasible and can identify the various contributors to the overall pollution burden. Lists of the PAHs found in the following five broad categories have characterized the variety of sources and identified some of the major contributors: heat and power generation, refuse-burning, industrial processes, motor vehicles, and natural sources.

In the 1978 review of the sources of PAHs, Baum,[4] using data assembled by EPA in 1974, estimated that 97% of the BaP emitted in the United States could be attributed to stationary fuel combustion. The major contributors were the inefficient combustion of coal in residential furnaces, coke ovens, and refuse fires. This is in close agreement with the NRC report Particulate Polycyclic Organic Matter, which stated that 90% of the annual nationwide BaP emission was attributable to coal- and wood-fired residential furnaces, coal-refuse fires, and coke production.

A wide range in concentrations of BaP (0.1-388 ng/m^3) has been reported by Colucci and Begeman[10] for U.S. and foreign cities (see Table 3-9). These results are for measurements taken between 1952 and 1966. The objective of the authors was to study BaP concentrations in the atmosphere in relation to automobile traffic. They used tracers to identify automotive and nonautomotive sources and calculated correlation coefficients of BaP with CO (a motor-vehicle tracer) as 0.65, with lead (a gasoline-vehicle tracer) as 0.74, and with vanadium (an oil tracer) as 0.54.* The seasonal variations show that the concentrations were highest in fall and winter and lowest in spring and summer. The winter vanadium concentrations were twice the summer concentrations; that indicates that the higher amount of BaP was attributable to combustion of residual fuels used for heating or to the lower inversion heights prevalent during cold weather.

The concentrations of BaP in England, Italy, Norway, Sweden, and Germany, as shown in Table 3-10 for 1953-1964, were given in the report by Louw,[60] for the purpose of comparing the findings in South Africa. The concentrations of BaP ranged between 5 and 49 ng/m^3. One sample, taken near a road-tarring operation, was extremely high, 1,113 ng/m^3.

In Ontario, Canada, five locations were sampled for BaP by Katz et al.[46] from April 1975 through March 1976. The highest concentration was observed in Hamilton (3,498 ng/m^3), and the lowest in Sudbury (111 ng/m^3); the latter was attributed to the electrostatic precipitators in use at the nickel-copper smelter 5 mi away. The concentrations are given in Table 3-11.

A study was conducted in Karlsruhe, Germany, to determine the relative amounts of BaP from residential heating systems and automobile traffic (see Figure 3-10). The concentrations ranged from 0.1 ng/m^3 (at the low-traffic Municipal Garden) to 28 ng/m^3 (at a railroad underpass). With lead as the tracer, it was determined that the highest concentration caused by automobiles was in the underpass. The low concentration in the

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*The correlations indicate that 42-55% of the variation in BaP concentrations is related to motor-vehicle tracers and 29% to stationary oil-burning.

Municipal Garden during summer was attributed to deposition of airborne particles on leaves, trees, and shrubs. During the winter, the increase in the concentration of BaP was attributed to increased residential heating. The air samples taken at Karlsruhe Nuclear Research Center, 11 km (by air) north of the city, had the lowest concentrations, except for those in the Garden during May and June.

BaP was determined in four locations around the industrial city of Essen, Germany, by Grimmer et al.,[28] from October 1978 to March 1979. There were four sampling sites at each of five locations. The authors concluded that the concentrations of BaP in ambient air varied by a factor of more than 10 from one station to another during the cold-weather heating period. Thus, they did not give any average values; the approximate ranges of concentration at each location were as follows: 1-75 ng/m^3 in an area that used hand-stoked coal-heating in residences, 1.5-21 ng/m^3 in an area with oil-heating only, 10-100 ng/m^3 in a tunnel with car traffic, 15-210 ng/m^3 in an area with coke ovens, and 1-75 ng/m^3 in an area described as rural, outside the city.

Two very thorough studies of the PAH content of Los Angeles air have been made by Gordon and Bryan[26] and Gordon.[25] The earlier study was of four locations in the Los Angeles basin (see Figure 3-11), and the latter included 13 sampling locations (see Figure 3-12). Analyses were performed for 14 PAHs, including BaP, sampled over the course of a year. From the relationship between meteorology, traffic density, and PAH concentrations, the authors concluded that most (at least 60%) of the PAHs was contributed by automobile traffic, but that the concentrations were lower than in many other cities. This result was expected, because of the extensive use of natural gas and hydrothermal energy in the West and the nonuse of coal in Los Angeles. The warm climate also limits wood-burning in fireplaces. The Colucci and Begeman[11] results demonstrated much higher ambient BaP concentrations in urban areas that depend extensively on coal, oil, and wood combustion. They determined that the automotive contribution to Detroit ambient BaP was only 5-42%, with typical BaP concentrations 3 times as high as in Los Angeles.

TABLE 3-1

The Effect of Adsorption on Carrier Particles on the Distribution of PAHs between Gaseous and Particulate Phases

PAH (Number of Aromatic Rings)	Vapor Pressure at 25°C, torr[a]	Equilibrium Gas Phase Concentration, at 25°C, ng/m^{3}[b]	Measured Ambient Air Concentraction, ng m^{3}[c]		
			Gas Phase[d]	Particulate Phase	P/G Ratio
Naphthalene (2)	8.2×10^{-2}	--	All	None	0
Anthracene (3)	2×10^{-4}	--	44.7	1.21	0.03
Phenanthrene (3)	6.8×10^{-4}	--			
Benz[a]anthracene (4)	1.1×10^{-7}	1.03×10^{3}	3.87[e]	12.2[e]	3.15[e]
Pyrene (4)	6.8×10^{-7}	74×10^{3}	3.36	1.64	0.49
Benzo[k]fluoranthene	--	16	2.0[f]	23.1[f]	11.5[f]
Benzo[a]pyrene (5)	5.5×10^{-9}	85			
Benzo[e]pyrene (5)	5.5×10^{-9}	85	2.69	20.1	7.5
Perylene (5)	--	--			
Benzo[ghi]perylene (6)	1.0×10^{-10}	1.6	--	--	--
Coronene (7)	1.5×10^{-12}	0.024	None[g]	All[g]	--

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[a] Data from Santodonato *et al.*[78]

[b] Data from Pupp *et al.*[76]

[c] Data from Cautreels and Van Cauwenberghe.[8] Samples collected in Antwerp, Belgium, Oct. 1976; average temperature, 10.2°C.

[d] Includes particulate PAH loss from filter at sampling linear airflow rate of 0.43 m/s.

[e] Sum of benz[e]anthracene and chrysene.

[f] Sum of benzo[a]fluoranthene and benzo[b]fluoranthene.

[g] Data from Miguel and Friedlander.[64]

TABLE 3-2

Size Distribution of Benzo[a]pyrene and Coronene in Pasadena, California, in Ambient Air[a]

Aerodynamic diameter, μm	Concentration, pg/m³							
	Oct. 25-28, 1976		Dec. 14-17, 1976		Feb. 1-4, 1977		Mar. 1-6, 1977	
	BaP	Cor	BaP	Cor	BaP	Cor	BaP	Cor
0.05-0.075	30	431	83	1,200	72	800	69	387
0.075-0.12	196	1,390	523	4,220	431	2,850	139	593
0.12-0.26	79	460	208	907	238	920	65	206
0.26-0.50	28	205	67	113	90	307	28	65
0.5-1.0	36	166	81	218	83	227	26	70
1.0-2.0	16	107	44	<45	39	93	16	50
2.0-4.0	16	118	30	<45	32	93	14	45
>4	21	136	23	<45	28	80	12	45

---

[a] Data from Miguel and Friedlander.[64]

TABLE 3-3[a]

PAH fluxes (in ng· cm^{-2}· yr^{-1}) to sediments from five remote sites in the northeastern United States for three age intervals: present, approximately 1950, and 1900; and to sediments from three urban sites for 1940 to the present.

Remote Sites	Interval	In Situ Density	Phen.	Anth.	C_1 Phen.	Fluo.	Pyr.	B[a]A	Chry.+ Tri.	B[e]P	B[a]P
Lake Superior, 0.02 cm/yr	1955-now	0.55	0.3	0.03	0.3	1	0.6	0.3	1	0.9	0.4
	1930-1955	0.55	0.2	0.02	0.2	0.9	0.5	0.3	1	0.9	0.3
	1870-1920	0.55	0.06	0.004	0.08	0.2	0.1	0.07	0.3	0.2	0.07
Isle Royale, 0.09 cm/yr	1974-now	0.33	0.4	0.01	0.5	0.4	0.3	0.2	0.8	0.8	0.2
	1951-1955	0.32	1	0.05	2.5	1	0.7	0.2	0.7	0.9	0.2
Somes Sound, 0.1 cm/yr	1960-now	0.43	2	0.2	1	5	4	2	2	2	2
	1940-1960	0.52	4	0.4	4	8	5	3	4	2	2
	1880-1940	0.51	0.4	<0.02	<0.2	0.4	0.4	< 0.05	< 0.2	0.2	0.2
Hadlock Lower Pond, 0.07 cm/yr	1950-now	0.12	2	0.1	2	4	3	0.6	1	0.8	0.6
	1920-1950	0.11	0.3	0.04	--	0.3	0.2	0.04	0.1	0.1	0.06
Coburn Mtn. Pond, 0.3 cm/yr	1975-now	0.036	2	0.2	4	4	3	0.7	2	2	0.7
	1943-1947	0.057	8	0.5	12	11	8	3	6	7	4
	1898-1901	0.057	0.8	0.07	1	0.5	0.3	0.2	0.9	0.4	0.1
Average	present	--	1	0.1	1.5	3	2	0.8	1.5	1.5	0.8
	1950	--	3	0.2	4.5	4	3	1.5	2.5	3	1.5
	1900	--	0.4	0.03	0.4	0.4	0.3	0.1	0.2	0.5	0.1

Table 3-3 (continued)

Urban Sites	Interval	In Situ Density	Phen.	Anth.	C_1 Phen.	Fluo.	Pyr.	B[a]A	Chry.+ Tri.	B[e]P	B[a]P
Boston Outer Harbor, 0.1 cm/yr	1900-now	0.93	24	5.6	17	37	39	19	23	14	17
Buzzards Bay, Mass., 0.3 cm/yr	1940-now	0.3	18	2	--	53	48	37	37	--	140[b]
Pettaquamscutt River, 0.3 cm/yr	1940-now	0.16	46	5	36	93	93	42	42	--	130[b]
Average		--	30	4	25	55	55	30	35	~ 25	~30

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[a] Reprinted with permission from Gschwend and Hites.32

[b] Includes all $C_{20}H_{12}$ isomers except perylene.

TABLE 3-4

Concentration of Polycyclic Aromatic Hydrocarbons in Norway Aerosols[a]

PAH	Concentration in Aerosol in Air from: England, France, Feb. 20-21, 1976	Northern Eng., Scotland, Nov. 25-26, 1975	Northern Norway, Jan. 25-27, 1976	Stationary air, Southern Norway, Feb. 1, 1976
Phenanthrene	4.725	1.216	0.036	0.146
Anthracene		0.278	0.038	--
Methylphenanthrene/ anthracene	0.661	0.216	--	0.052
Fluoranthene	6.637	3.965	0.171	0.324
Dihydrobenzo[a&b]-fluorenes	0.874	0.363	0.032	0.032
Pyrene	4.864	3.293	0.135	0.286
Benzo[a]fluorene	0.815	0.318	0.021	0.026
Benzo[b]fluorene	0.571	0.149	0.117	0.148
1-Methylpyrene	0.147	0.099	0.009	0.009
Benzo[c]phenanthrene	1.021	0.957	0.038	0.108
Benz[a]anthracene	0.585	0.740	0.041	0.073
Chrysene/triphenylene	1.756	3.269	0.099	0.194
Benzo[b&k]fluoranthene	4.312	4.013	0.083	0.464
Benzo[e]pyrene	1.191	2.635	0.066	0.135
Benzo[a]pyrene	0.965	2.053	0.059	0.098
Perylene	0.090	0.191	Trace	0.011
Ideno[1,2,3-cd]pyrene	1.306	1.920	0.062	0.144
Benzo[ghi]perylene	1.142	1.971	0.064	0.140
Anthanthrene	0.225	0.423	0.007	0.022
Coronene	0.212	0.183	--	0.020
Total	32.099	28.252	1.078	2.432

[a] Data from Lunde and Bjorseth[61] and Bjorseth *et al*.[6]

TABLE 3-5

Heterogeneous Photo-oxidation and Ozonolysis Half-Lives of PAHs on TLC Plates[a]

	Half-Life, h		
PAH	Ozonolysis in Dark (O_3 = 0.2 ppm)	Photo-oxidation (quartz-lamp irradiation in air)	Photo-oxidation and Ozonolysis
Anthracene	1.23	0.2	0.15
Benz[a]anthracene	2.88	4.2	1.35
Dibenz[ah]anthracene	2.71	9.6	4.8
Dibenz[ac]anthracene	3.82	9.2	4.6
Pyrene	15.72	4.2	2.75
Benzo[a]pyrene	0.62	5.3	0.58
	0.4[b]	--	0.2[b]
	0.3[c]	--	0.08[c]
Benzo[e]pyrene	7.6	21.1	5.38
Benzo[b]fluoranthene	52.7	8.7	4.2
	10.8[b]	--	3.6[b]
	2.9[c]	--	1.9[c]
Benzo[k]fluoranthene	34.9	14.1	3.9
	13.8[b]	--	3.1[b]
	3.3[c]	--	0.9[c]

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[a] Data from Lane and Katz[57] and Katz *et al.*[45]

[b] O_3 = 0.7 ppm.

[c] O_3 = 2.3 ppm.

TABLE 3-6

Reaction of Adsorbed PAHs with Nitrogen Dioxide

PAH	$PAH-NO_2$ Reaction Half-life,[a] d	Nitro Derivatives Identified	Nitro Derivative Yield Measured	Effect of Substrate Investigated
Phenanthrene	30	Mononitro, isomer not specified[b]	b	--
Anthracene	--	9-Nitro[c,d]	--	c
Fluoranthene	27	3-Nitro[b] and 8-nitro[b]	b	--
Chrysene	26	6-Nitro[b,c]	b	c
Pyrene	14	1-Nitro[b,c,e] and dinitro[e]	b,c,e	c,e
Benzo[a]pyrene	7	6-Nitro,[c,f] 1-nitro, 3-nitro,[f] and dinitro[c]	c,f	c
Benzo[e]pyrene	24	--	--	--
Perylene	--	3-Nitro[d,f]	f	--
Benz[a]anthracene	11	--	--	--
Benzo[ghi]perylene	8	--	--	--
Anthanthrene	3.7	--	--	--
Fluorene	--	2-Nitro[b]	b	--
Coronene	29	--	--	--
Carbazole	--	Two unspecified isomers[b]	b	--

---

[a] Data from Butler and Crossley.[7]

[b] Data from Tokiwa et al.[84]

[c] Data from Jäger and Hanus.[42]

[d] Data from Gundel et al.[33]

[e] Data from Hughes et al.[37]

[f] Data from Pitts et al.[75]

TABLE 3-7

Yields of Nitro PAHs as Function of Substrate[a]

	Nitro-PAH yield, µg/100 µg of PAH			
	1-Nitropyrene			
Substrate	Dark	UV Light	Daylight	6-Nitro-BaP, Daylight
Carbon	0.45	0.38	0.40	Trace
Alumina, deactivated	2.4	2.3	2.6	7.9
Alumina, activated	1.9	2.0	2.8	8.2
Fly ash	36.8	41.8	57.3	15.8
Silica gel	--	--	112.5	25.5

---

[a] Data from Jäger and Hanus.[42]

TABLE 3-8

Influence of Substrate on Photo-Oxidation of PAH

PAH	Half-life, h, Pure PAH on Cellulose TLC Plate[a]	% Destruction in 48 h[b] Pure PAH on Whatman Paper	Adsorbed on Soot	Loss, %, Adsorbed on Fly Ash[c]
Anthracene	0.2	--	--	17-26
Benz[a]anthracene	4.2	--	--	--
Dibenz[ab]anthracene	9.6	--	--	--
Dibenz[ac]anthracene	9.2	--	--	--
Pyrene	4.2	42	1	5-13
Benzo[a]pyrene	5.3	22	10	9-17
Benzo[e]pyrene	21.1	--	7	--
Benzo[b]fluoranthene	8.7	--	--	--
Benzo[k]fluoranthene	14.1	--	--	--
Anthanthrene	--	44	5	--
Phenanthrene	--	60	--	0
Fluoranthene	--	24	4	0
Benzo[ghi]perylene	--	0	0	--
Coronene	--	0	--	--
Chrysene	--	0	--	--

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[a] Data from Katz _et_ _al_.[45]

[b] Data from Falk _et_ _al_.[17]

[c] Data from Korfmacher _et_ _al_.[50] Different light sources (xenon, quartz, etc.). Times up to 100 h.

TABLE 3-9
Benzo[a]pyrene Content of Urban Air[a]

Location	Benzo[a]pyrene Content, ng/m^3			
	Spring	Summer	Fall	Winter
New York:				
Commercial	0.5-8.1	0.7-3.9	1.5-6.0	0.5-9.4
Freeway	0.1-0.8	0.1-0.7	3.3-3.5	0.7-1.3
Residential	0.1-0.6	0.1-0.3	0.6-0.8	0.5-0.7
Detroit:				
Commercial	7.2	--	--	5.0-17.0
Freeway	--	4.0-6.0	3.4-7.3	9.2-13.7
Residential	--	0.2	--	0.9-1.8
Atlanta	2.1-3.6	1.6-4.0	12-15	2.1-9.9
Birmingham	6.3-18	6.1-10	20-74	23-34
Detroit	3.4-12	4.1-6.0	18-20	16-31
Los Angeles	0.4-0.8	0.4-1.2	1.2-13	1.1-6.6
Nashville	2.1-9.0	1.4-6.6	30-55	25
New Orleans	2.6-5.6	2.0-4.1	3.6-3.9	2.6-6.0
Philadelphia	2.5-3.4	3.5-19	7.1-12	6.4-8.8
Pittsburgh	--	0-23	2.9-37	8.2
San Francisco	0.8-0.9	0.2-1.1	3.0-7.5	1.3-2.4
Hamburg, Germany	14.72	10-26	66-296	94-388
London, England	25-48	12-21	44-122	95-147
Sheffield, England	20-44	21-33	56-63	64-78
Cannock, England	4-16	6-11	27-31	27-32
London:				
Traffic	20	11	57	68
Background	11	1	38	42
Milan, Italy	12	3	25	150
Copenhagen, Denmark	6	5	14	15
Prague, Czechoslovakia	--	13-36	--	53-145
Budapest, Hungary	--	17-32	--	72-141
South Africa:				
Pretoria	--	10	--	22-28
Johannesburg	--	--	--	22-49
Durban	--	--	--	5-28
Osaka, Japan				
Commercial	5.7	1.7	9.4	14
Residential	3.3	1.4	3.8	6.7
Sidney, Australia	0.6-2.4	0.6-1.8	2.5-7.4	3.8-8.2

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TABLE 3-10

Benzo[a]pyrene Concentrations in Pretoria, Johannesburg, Durban, and Other Large Cities[a]

			Benzo[a]pyrene Concentration, ng/m^3	
Country	City	Period of Sampling	Uncorrected	Corrected for Benzo[k]fluoranthene
S. Afr.	Pretoria	26-27 Aug. 1963[b]	67	22
		23-24 Sept. 1963[b]	83	28
		20-21 Jan. 1964[c]	31	10
	Johannesburg	27-28 Apr. 1964[b]	65	22
		4-5 May 1964[b]	141	47
		18-19 May 1964[b]	146	49
		12 May 1964[b,d]	3,340	1,113
	Durban	10-11 June 1964[b]	42	14
		11-12 June 1964[b]	16	5
		16-17 June 1964[b]	83	28
England	Merseyside and other northern localities	(i) Jan.-Dec. 1958	11	108
		1954-1957[b]	17	166
		(ii) 1954-1957[b]	6	37
	Salford	Nov. 1952-Mar. 1953	197	290
	Sheffield	June 1949-Apr. 1950	20	28
	Cannock	July 1949-June 1950	4	32
Italy	Milan	Jan.-Oct. 1958	3	231
Norway	Oslo	Feb.-Dec. 1955	0.86	15.2
Sweden	Stockholm	Mar.-July 1960	1.1	10.0
Germany	Hamburg	Sept. 1961-Apr. 1963	10.1	388

---

[a] Adapted from Louw.[60]

[b] Winter.

[c] Summer; determined by direct chromatography of the cyclohexane-soluble fraction of sample on thin-layered alumina.

[d] Road-tarring operation.

Table 3-11

Seasonal Concentrations of Benzo[a]pyrene in Air of Ontario Cities
April 1975-March 1976[a]

Location	Apr.-Jun. 1975		Jul.-Sept. 1975		Oct.-Dec. 1975		Jan.-Mar. 1976	
	ng/m^3	μg/g[b]	ng/m3	μg/g[b]	ng/m3	μg/g[b]	ng/m3	μg/g[b]
South Sarnia	338	5.5	114	2.4	596	11.4	190	7.0
Hamilton	1,404	9.6	2,351	16.9	3,498	50.6	1,934	23.1
Toronto (Kennedy at Lawrence, suburban)	657	8.7	408	6.2	729	11.7	814	5.9
Toronto (Bathur Street at 401)	789	8.8	1,047	11.0	1,674	20.2	720	9.2
Sudbury (5 mi from nickel-copper smelter)	175	5.4	111	2.6	342	15.3	444	19.0

---

[a] Adapted from Katz *et al*.[46]

[b] μg/g = μg of PAH per gram of total particles--same as parts per million.

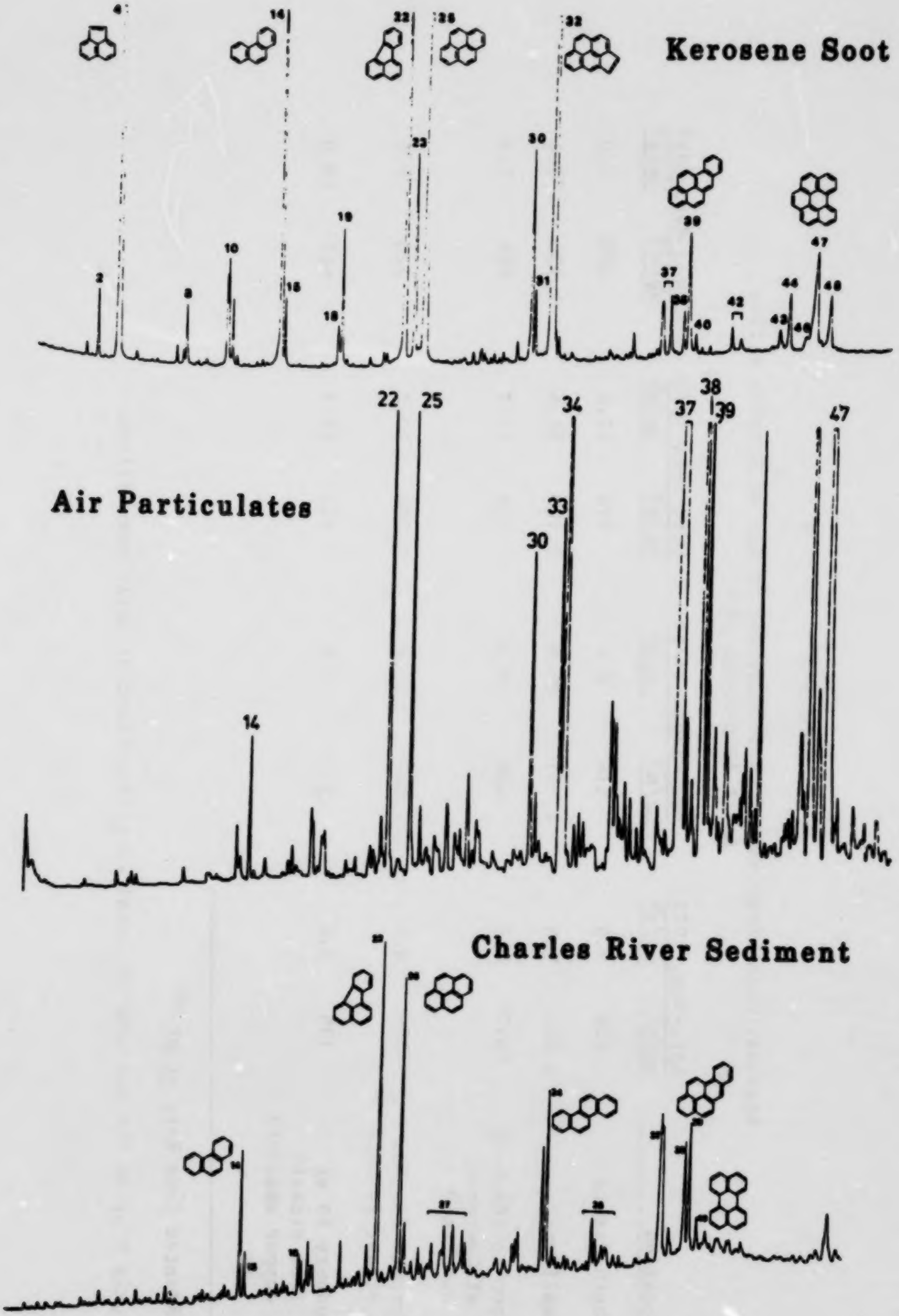


FIGURE 3-1. Gas chromatograms of PAH mixtures obtained from (top) soot from kerosene flame, (middle) urban air particles, and (bottom) sediment of Charles River in Boston.

Peak Identifications:

2	Biphenyl
4	Acenaphthylene
8	Fluorene
10	$C_{14}H_8$
14	Phenanthrene
15	Anthracene
18	Methylphenanthrene
19	4H-Cyclopenta[def]-phenanthrene
22	Fluoranthene
23	Benz[e]acenaphthylene
25	Pyrene
27	Methylfluoranthene
30	Benzo[ghi]fluoranthene
31	$C_{18}H_{10}$ (unknown)
32	Cyclopenta[cd]pyrene
33	Benz[a]anthracene
34	Chrysene
35	Methylchrysene
37	Benzofluoranthene
38	Benzo[e]pyrene
39	Benzo[a]pyrene
40	Perylene
42	$C_{21}H_{12}$ (unknown)
43	$C_{21}H_{12}$ (unknown)
44	Indeno[1,2,3-cd]pyrene
46	Dibenz[ac]anthracene
47	Benzo[ghi]perylene
48	Anthanthrene

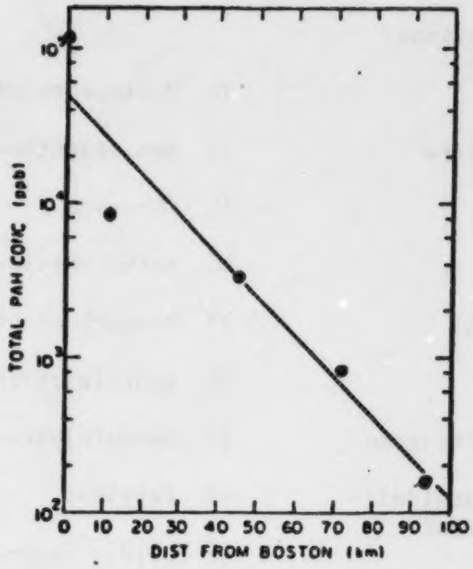


FIGURE 3-2. Total PAH concentrations vs. distance from Boston for Massachusetts Bay samples. Reprinted with permission from Windsor and Hites;[90] copyright 1979, Pergamon Press, Ltd.

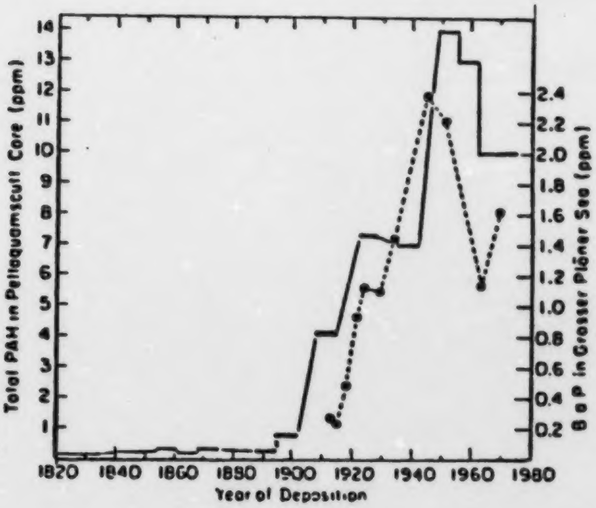


FIGURE 3-3. Total PAH abundance in the various Pettaquamscutt River sediment core sections vs. date of deposition (solid line, left scale); BaP abundance in Gosser Ploner Sea vs. date of deposition (dotted line, right scale). Reprinted with permission from Hites *et al.*;[35] copyright 1980, Pergamon Press, Ltd.

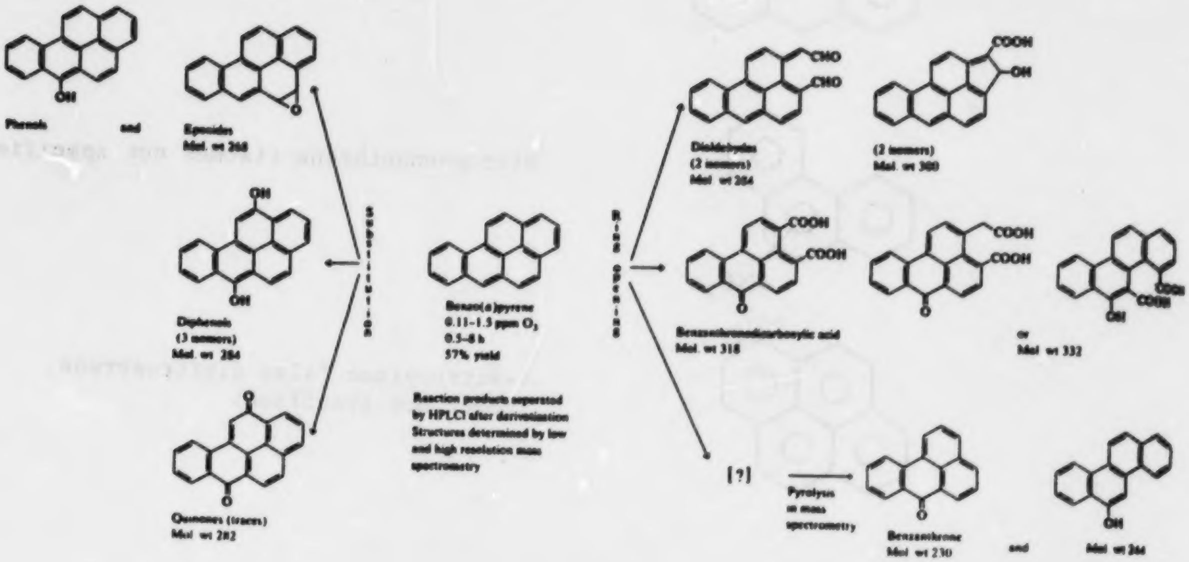

OH
Phenols
and
O
Epoxides
Mol. wt 268
OH
OH
Diphenols
(3 isomers)
Mol. wt 284
O
O
Quinones (traces)
Mol. wt 282
Substitution
Benzo(a)pyrene
0.11–1.5 ppm O_3
0.5–8 h
57% yield
Reaction products separated
by HPLC after derivatization
Structures determined by low
and high resolution mass
spectrometry
Ring opening
CHO
CHO
Dialdehydes
(2 isomers)
Mol. wt 284
COOH
OH
(2 isomers)
Mol. wt 300
COOH
COOH
O
Benzanthronedicarboxylic acid
Mol. wt 318
COOH
COOH
O
COOH
COOH
OH
or
Mol. wt 332
[?]
Pyrolysis
in mass
spectrometry
O
Benzanthrone
Mol. wt 230
and
OH
Mol. wt 244

FIGURE 3-4. Major reaction pathways and tentative structure of products of gas-phase ozonolysis of BaP. Most structures given as examples of possible isomers. Reprinted with permission from Van Vaeck _et_ _al_.;[86] copyright 1980, John Wiley & Sons Ltd.

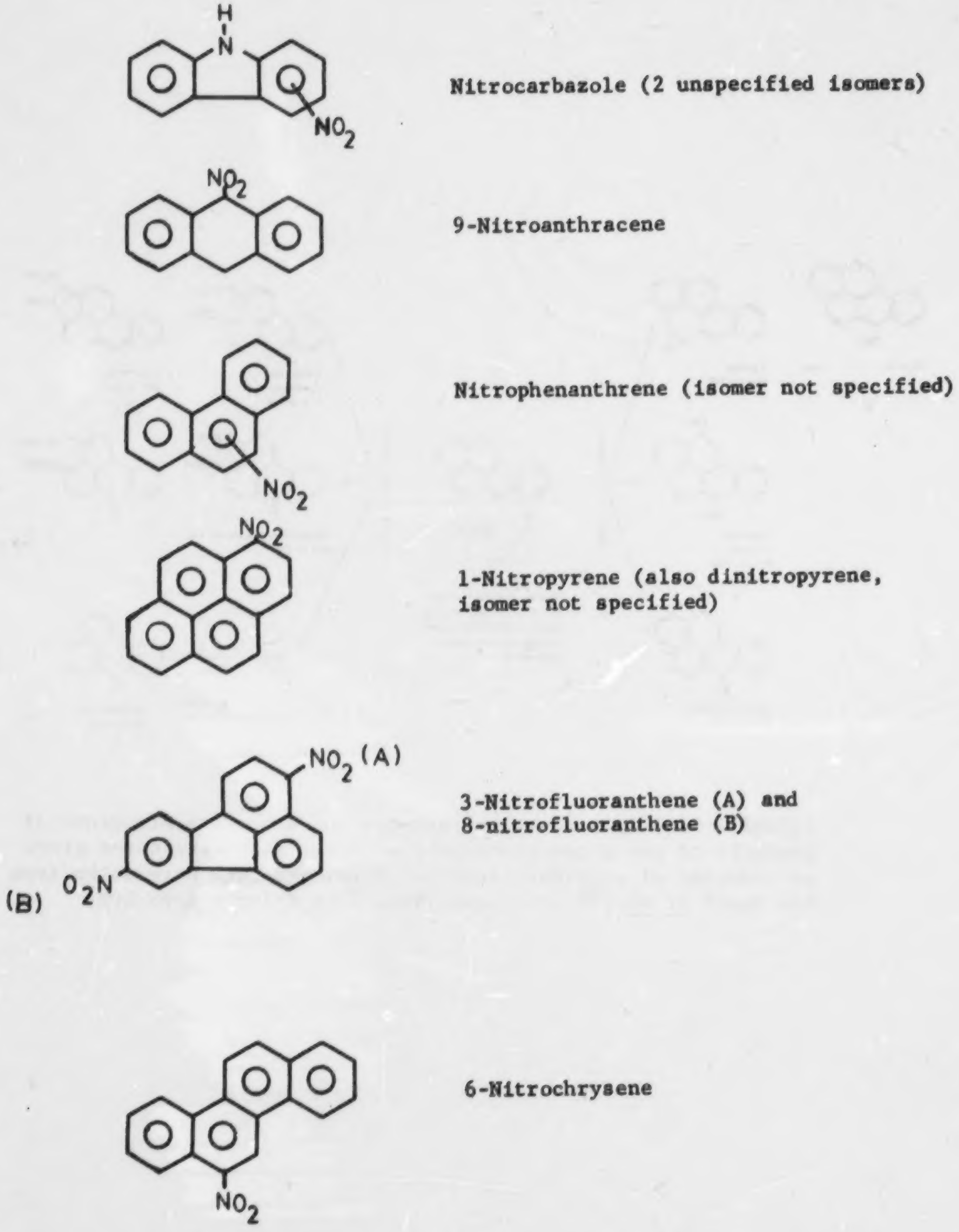
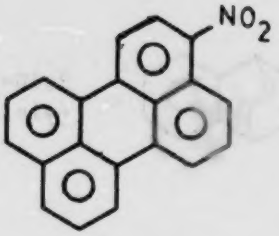
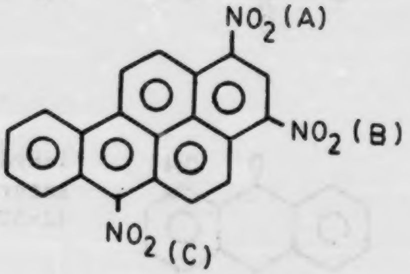

Nitrocarbazole (2 unspecified isomers)
9-Nitroanthracene
Nitrophenanthrene (isomer not specified)
1-Nitropyrene (also dinitropyrene, isomer not specified)
3-Nitrofluoranthene (A) and 8-nitrofluoranthene (B)
6-Nitrochrysene
H
N
NO2
NO2 (A)
(B) O2N

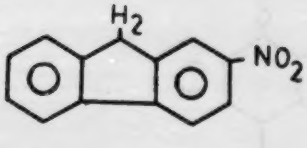
FIGURE 3-5. Nitro products identified in heterogeneous reactions of PAH with nitrogen dioxide.



3-Nitroperylene


NO_2 (A)
NO_2 (B)
NO_2 (C)

1-Nitrobenzo[a]pyrene (A), 3-nitrobenzo[a]pyrene (B), and 6-nitrobenzo[a]pyrene (C)--also dinitro-BaP, isomer not specified



2-Nitrofluorene

FIGURE 3-5. (continued)

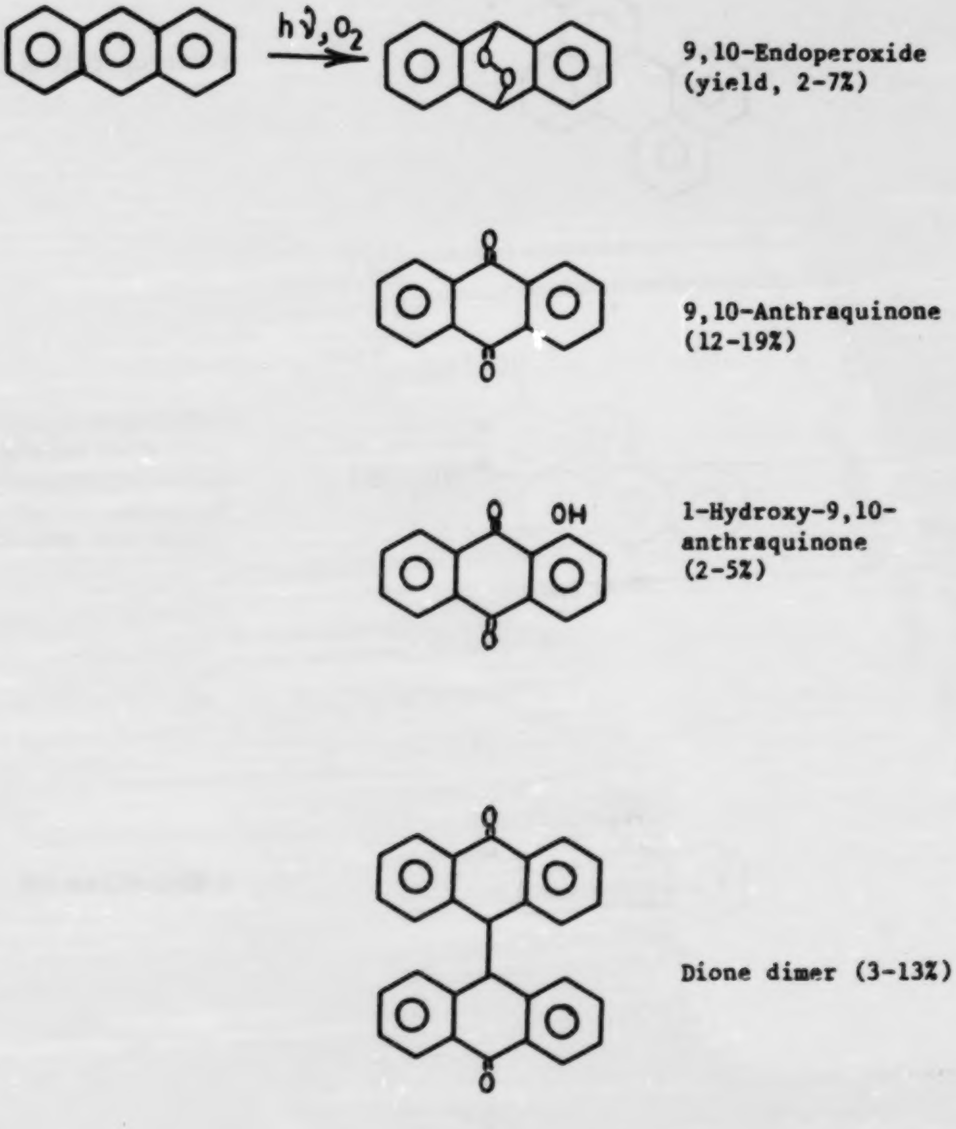

hν, O2
9,10-Endoperoxide
(yield, 2-7%)
9,10-Anthraquinone
(12-19%)
OH
1-Hydroxy-9,10-
anthraquinone
(2-5%)
Dione dimer (3-13%)

FIGURE 3-6. Products of heterogeneous photooxidation of anthracene on atmospheric particulate matter. Reprinted with permission from M. A. Fox and S. Olive, Science 205:582-583, 1979;[21] copyright 1979 by the American Association for the Advancement of Science.

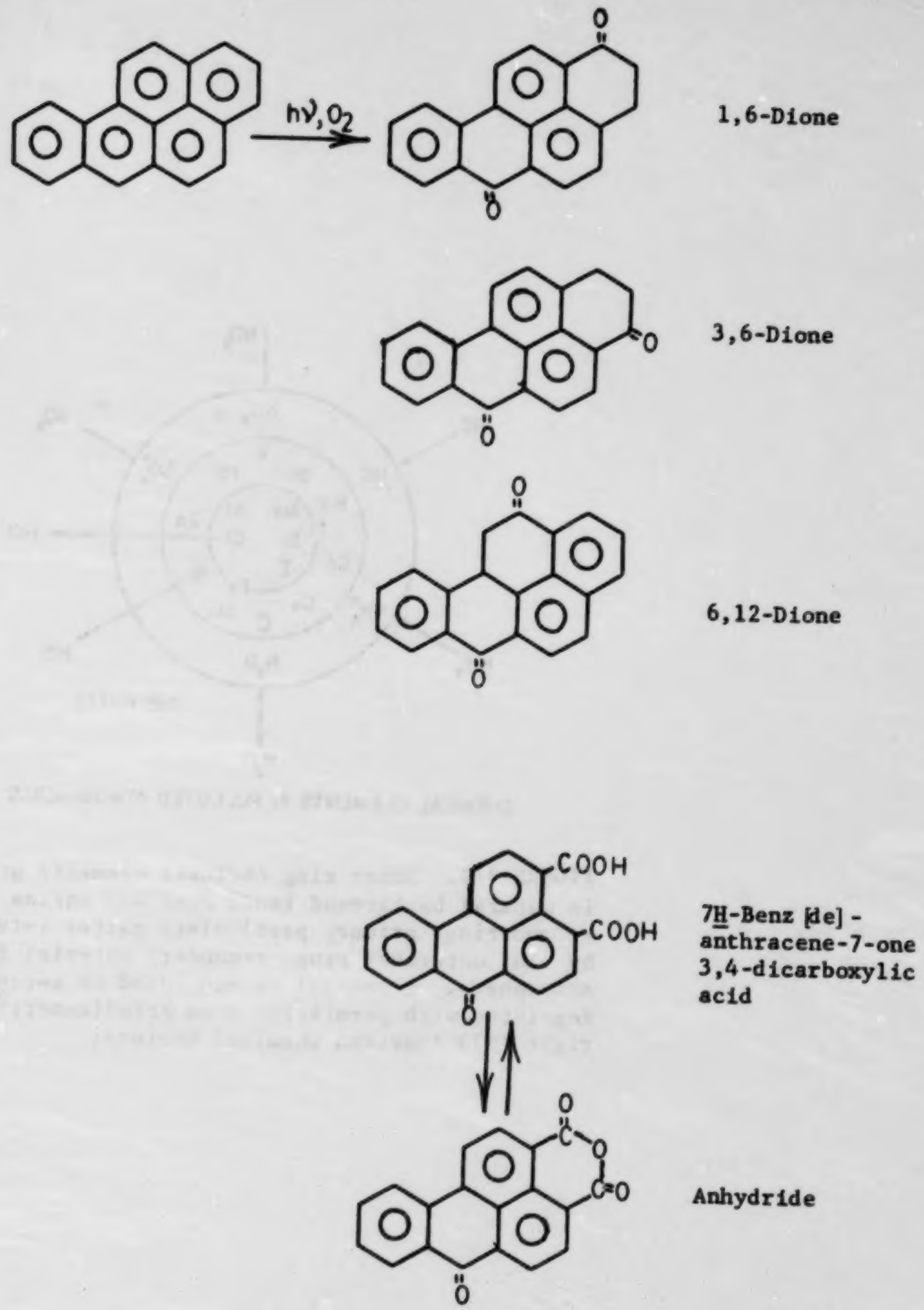

hν, O2
1,6-Dione
3,6-Dione
6,12-Dione
COOH
COOH
7H-Benz [de] -
anthracene-7-one
3,4-dicarboxylic
acid
Anhydride

FIGURE.3-7. Products identified in heterogeneous photooxidation of BaP. Reprinted with permission from Tebbens et al.[81]

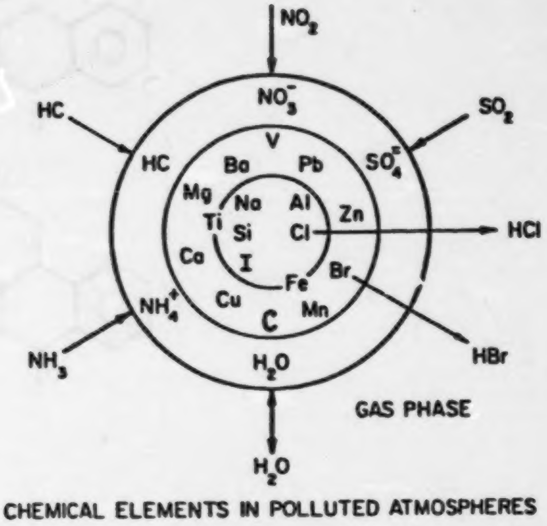

NO_2
NO_3^-
HC
HC
SO_2
$SO_4^=$
V
Ba
Pb
Mg
Na
Al
Zn
Ti
Si
Cl
HCl
Ca
I
Fe
Br
Cu
C
Mn
NH_4^+
NH_3
HBr
H_2O
GAS PHASE
H_2O
CHEMICAL ELEMENTS IN POLLUTED ATMOSPHERES

FIGURE 3-8. Inner ring encloses elements present in natural background (soil dust and marine aerosol); second ring, primary particulate matter introduced by man; outermost ring, secondary material formed in atmosphere. Elemental carbon added to second ring. Reprinted with permission from Friedlander;[22] copyright 1973 American Chemical Society.

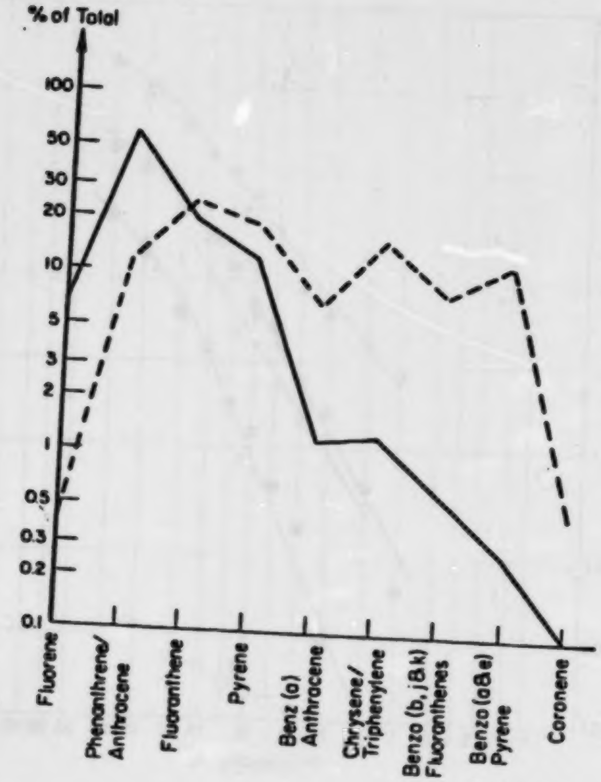


FIGURE 3-9. Parent PAH profile of PAH in particulate matter from aluminum plant (dashed line) and Soderberg paste plant (solid line). Soderberg paste plant is one in which electrodes used in production of aluminum are made of anthracite or of anthracite and petroleum coke. During baking of these electrodes, volatile components are produced from anthracite ore base. Reprinted with permission from Bjorseth;[6] copyright Ann Arbor Science Publishers, Inc.

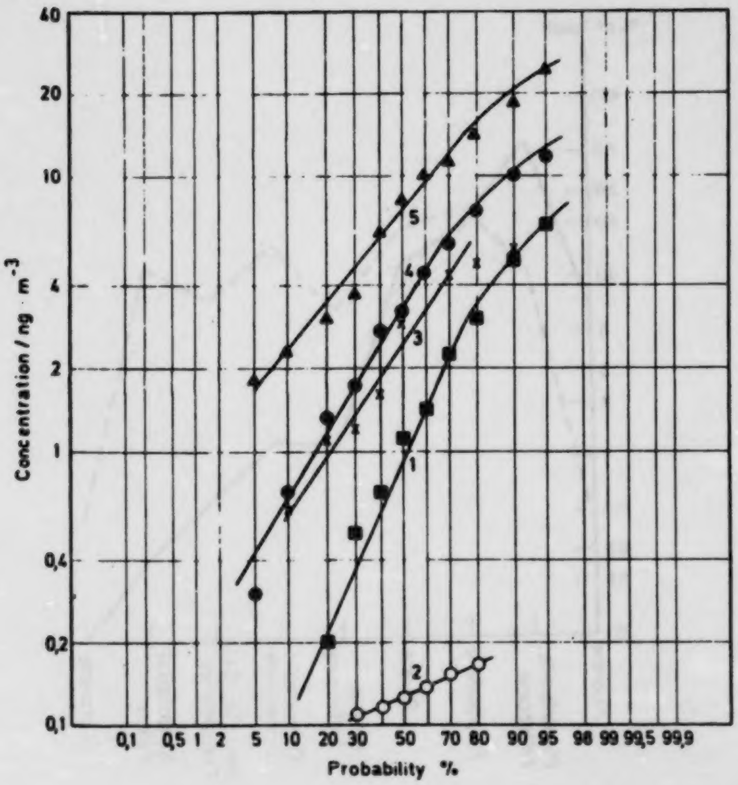


FIGURE 3-10. Frequency distribution of airborne BaP concentration at different measuring sites in area of Karlsruhe. 1, nuclear research center, Karlsruhe, November 1974-March 1975; 2, municipal garden, May-June 1975; 3, railroad underpass, May-June 1975; 4, municipal garden, October 1975-March 1976; 5, railroad underpass, October 1975-March 1976.

Component	Location 1	2	3	4
Total particulate mass, μg/m³	215	131	102	40
Benzene solubles, μg/m³	21.7	13.2	8.3	2.6
Lead, μg/m³	5.35	2.50	1.97	0.50
Traffic density × 10^{-3}, vehicle mi/mi²/day	200	130	95	8
PAH, ng/m³				
Coronene	6.4	3.2	2.8	0.20
Pyrene	2.0	1.4	3.8	0.18
Fluoranthene	1.9	0.8	3.4	0.12
Benz(*a*)-anthracene	1.1	0.8	3.1	0.04
Chrysene	2.6	1.6	3.8	0.04
Benzo(*e*) pyrene	3.0	1.8	3.2	0.09
Benzo(*a*) pyrene	1.1	0.5	3.5	0.03
Benzo(*b*)-fluoranthene	1.6	0.9	1.8	0.09
Benzo(*j*)-fluoranthene	0.6	0.3	0.8	0.01
Benzo(*k*)-fluoranthene	0.8	0.3	1.3	0.03
Perylene	0.5	0.3	1.2	0.01
Anthanthrene	0.4	0.2	1.1	0.01
Benzo(*ghi*)-perylene	9.2	4.2	7.1	0.21
Indeno(1,2,3-*cd*)-pyrene	1.2	0.4	0.3	0.03

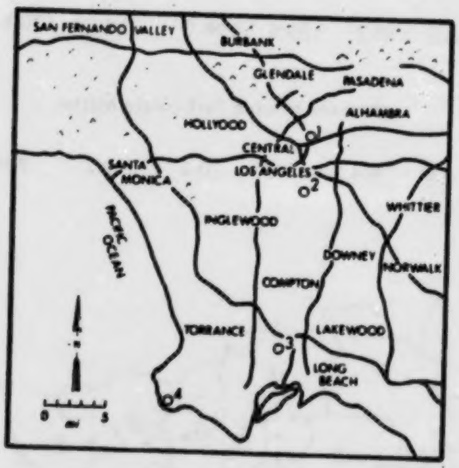

SAN FERNANDO VALLEY
BURBANK
GLENDALE
PASADENA
ALHAMBRA
HOLLYWOOD
CENTRAL
LOS ANGELES
1
2
SANTA MONICA
PACIFIC OCEAN
WHITTIER
INGLEWOOD
DOWNEY
NORWALK
COMPTON
TORRANCE
LAKEWOOD
3
LONG BEACH
4
N
0
mi
5

FIGURE 3-11. Components in Los Angeles airborne particles. Composite June 1971-June 1972. Map shows approximate location of sampling sites. Reprinted with permission from Gordon and Bryan;[26] copyright 1973 American Chemical Society.

PAH	Area												
	1	2	3	4	5	6	7	8	9	10	11	12	13
PYR	0.41	0.46	0.37	0.49	0.47	*0.76*	*0.84*	*0.67*	0.34	0.34	0.33	0.42	0.29
	0.36	0.50	0.40	0.60	0.48	0.61	0.46	0.33	0.39	0.38	0.30	0.44	0.30
FLT	0.28	0.32	0.20	0.33	0.30	0.50	*0.61*	*0.55*	0.25	0.27	0.21	0.30	0.22
	0.23	0.32	0.26	0.39	0.31	0.40	0.30	0.21	0.25	0.25	0.19	0.28	0.19
BAA	*0.18*	0.18	0.15	*0.26*	*0.21*	*0.44*	*0.23*	*0.24*	0.10	0.12	0.11	*0.17*	0.11
	0.13	0.18	0.15	0.22	0.18	0.23	0.17	0.12	0.15	0.14	0.11	0.16	0.11
CHY	0.62	0.68	0.36	0.65	0.76	0.92	*1.02*	0.68	0.53	0.42	0.38	0.66	0.45
	0.44	0.60	0.49	0.72	0.59	0.75	0.57	0.40	0.48	0.46	0.36	0.53	0.37
BEP	0.81	1.01	0.73	1.06	1.00	1.34	1.22	0.88	0.92	0.77	0.65	0.89	0.70
	0.75	1.04	0.85	1.26	1.02	1.30	0.98	0.69	0.83	0.80	0.63	0.92	0.63
BAP	0.47	*0.63*	0.41	0.56	0.54	*0.77*	*0.76*	*0.53*	0.41	0.35	0.24	0.38	0.27
	0.32	0.45	0.36	0.54	0.44	0.56	0.42	0.30	0.36	0.35	0.27	0.40	0.27
BJF	0.17	*0.25*	0.17	0.18	0.23	0.28	*0.26*	0.14	0.14	0.14	0.10	0.15	0.11
	0.13	0.19	0.15	0.22	0.18	0.23	0.17	0.12	0.15	0.14	0.11	0.16	0.11
BKF	0.16	*0.26*	0.16	0.21	*0.23*	*0.27*	*0.29*	*0.25*	0.14	0.16	0.11	0.19	0.11
	0.14	0.19	0.15	0.23	0.18	0.23	0.18	0.13	0.15	0.14	0.11	0.17	0.11
ANT	0.25	0.35	0.28	0.29	0.26	0.42	0.38	0.25	0.18	0.15	0.13	0.17	0.12
	0.18	0.25	0.21	0.31	0.25	0.32	0.24	0.17	0.20	0.20	0.15	0.22	0.15
GEE	2.86	3.78	3.02	4.33	3.84	*5.01*	*4.02*	2.67	2.99	3.05	2.31	3.41	2.32
	2.73	3.79	3.08	4.57	3.69	4.72	3.56	2.52	3.02	2.91	2.29	3.35	2.30
INP	1.10	1.22	1.11	1.89	1.55	2.05	*1.96*	1.18	1.33	1.18	0.90	1.48	1.01
	1.09	1.51	1.22	1.82	1.47	1.88	1.41	1.00	1.20	1.16	0.91	1.33	0.91
COR	1.83	2.54	2.06	3.06	2.47	3.16	2.38	1.69	2.02	1.95	1.53	2.24	1.54

Upper value in each pair = observed; lower value calculated using the average PAH/COR ratio for areas 3, 11, and 13. Italicized observed values exceed calculated values by at least three times the coefficient of variance among 3, 11, and 13.

Area	1	2	3	4	5	6	7	8	9	10	11	12	13
Automobile Traffic Density (ATD), 10^{-6} Mi/Da/Mi² (*5*)													
	0.75	1.2	1.5	1.5	2.0	1.2	0.65	0.4	0.8	1.05	0.95	0.95	0.9
Sample Yields, μg/m³													
Suspended Particulate Matter													
Geom. mean, year	84.2	87.2	74.5	85.5	85.8	109.5	128.0	118.8	106.8	82.4	66.1	83.9	79.0
Benzene-soluble Particulate Matter													
Geom. mean, year	7.7	9.6	6.5	9.0	8.4	11.3	10.8	9.2	7.9	7.3	5.3	6.8	5.9

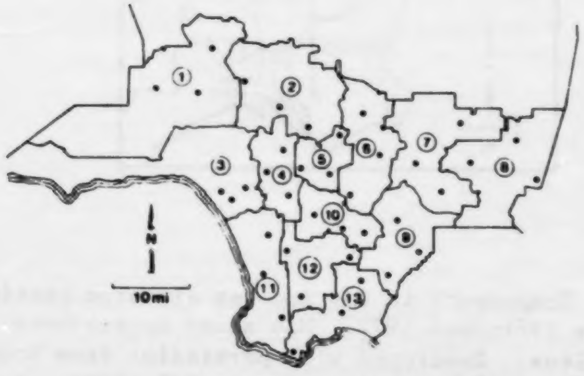


FIGURE 3-12. Observed PAH annual geometric mean concentrations, ng/m^3, and calculated on basis of patterns in coastal areas. Map shows approximate location of sampling sites. Reprinted with permission from Gordon;[25] copyright 1976 American Chemical Society.

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4

BIOLOGIC EFFECTS OF SMOKE, EMISSION, AND SOME OF THEIR PAH COMPONENTS

The environment is a major contributor to the development of a variety of pathologic conditions in humans. Indeed, Doll and Peto[43] have estimated that as much as 13% of all human deaths from cancer may be attributed to exposure to harmful polluting substances in our environment. The purposes of this chapter are to describe the biologic activity of various kinds of polluting emission and some of their PAH components and to attempt to relate the toxic impact of such emission to its content of specific PAHs. (See Chapter 3 for discussion of particle size and respiratory uptake, Chapter 6 for discussion of PAH transfer in tissues, and Chapter 9 for recommendations.) It considers biologic activity in bacteria, animal-cell systems, and intact animals, as well as the nature and advantages of some biologic models used in emission toxicology.

Hilado and colleagues[65-68] have reported considerable morbidity in experimental animals that were exposed to the products of combustion of hard woods, such as birch and oak, or soft woods, such as fir and pine; they noted no difference in toxicity between the products of these hard and soft woods. The problem of interpreting results related to wood is compounded by the presence of preservatives and other additives in the wood. It is often difficult to establish whether any observed toxicity is caused by the combustion products of the wood itself or of a contaminating additive. And it has not been established whether the PAHs generated during combustion contribute more to the observed toxicity than the gaseous products. Considerable additional work with subacute and chronic exposure is required to characterize toxicity, particularly in view of the current increase in such emission.

Toxicity has been measured in rats and mice intermittently exposed to diesel exhaust for periods up to 308 h.[110] The total cumulative particulate exposure varied from 7.75 to 1,310 mg/m^3-h. However, only minimal changes from normal were observed. Glutathione reductase and lactic acid dehydrogenase activities, which might serve as indicators of lung-cell damage, were increased in lavage fluid after 3 wk of exposure at the high dosage; although exposure was continued, they returned to normal by 6 wk. Neutral protease activity was increased in lavage fluid after 1 wk of exposure at the medium dosage (30.6 mg/m^3-h) and the high dosage, but returned to normal by the twelfth week of continued exposure. It is of interest that no alteration in cytochrome P_{450} activity was observed in either mouse or rat liver at any time in any group. After 12 wk of exposure at the highest dosage, an increase in the number of macrophages was seen in the lavage fluid.

TOXICITY TO SPECIFIC ORGANS AND ORGAN SYSTEMS IN ANIMALS

Manifestations of toxicity to specific organs and organ systems were detected in animals that were exposed to various kinds of emission.

PULMONARY FUNCTION

Many studies have been conducted in which animals were exposed to diesel-exhaust particles (generally 0.1-0.2 μm in size). Abraham et al.[1] reported little change in pulmonary resistance or in airway reactivity to a carbachol aerosol in conscious sheep exposed for 30 min to diesel-exhaust particles. Battigelli[7] exposed human volunteers for up to 1 h to diesel exhaust at total hydrocarbon concentrations of 2-6 ppm (comparable with the environment in railroad shops) and then measured pulmonary resistance as an index of function. With this rather insensitive assay of only relatively short duration, no changes in function were observed.

Mauderly et al.[110] measured tracheal mucociliary clearance of a ^{99m}Tc-macroaggregated albumin suspension that had been instilled intratracheally in rats 1 wk before exposure to diesel exhaust for 1, 6, or 12 wk. They also examined the morphology of the lung and trachea with scanning electron microscopy. In the group of animals that had been exposed at high dosages (cumulative particle exposure of 151, 822, or 1,310 mg/m^3-h, respectively, after 1, 6, or 12 wk of exposure), clearance of the suspension was increased after 1 wk; by 12 wk, it was below normal. In this group, a tendency toward reduced numbers of ciliated cells was noted. Furthermore, a dose-related increase in pulmonary macrophages was apparent. Many of these cells contained diesel particles as inclusions. No changes were seen in the morphology of the alveoli or airways. In the groups of animals that were exposed during the same times at lower dosages (30.6, 203, or 317 mg/m^3-h), a reduction in clearance was the more prevalent response. However, in the pulmonary function part of this study, in no group of exposed mice or rats was any significant alteration in pulmonary function observed. A similar lack of effect on pulmonary function after diesel-exhaust exposure of rats was reported by Pepelko[125] and by Gross.[58] In the study of Pepelko et al., rats were exposed for 20 h/d, 7 d/wk, for 28 d to a 1:4 raw or irradiated exhaust from a six-cylinder Nissan diesel engine. Gross exposed rats to diesel-exhaust particles at 1,500 μg/m^3 for 20 h/d, 5 d/wk, for up to 267 d, but suggested that a longer chronic exposure of the rats to the particles might result in lung disease.

Because guinea pigs are generally more susceptible to pulmonary lesions, they were similarly exposed to diesel-exhaust particles[5] for periods varying from 2 wk to 3 mo at 250-6,000 μg/m^3. As reported for other species, the number of pulmonary alveolar macrophages increased, and they tended to accumulate at the bronchoalveolar junctions. Occasional localization of the particles in alveolar Type I epithelial cells and sporadic increases in Type II cells were observed. However, all these morphologic changes would be classified as minimal with regard to pulmonary toxicity.

A different tack was taken by Mauderly *et al.*[110] and by Campbell *et al.*[17] to demonstrate an alteration in pulmonary function after exposure of rats or mice to diesel-exhaust particles. In the former study, exposed rats were inoculated with ^{32}P-labeled *Pseudomonas aeruginosa* at the oropharynx, and the killing and clearance of these organisms were ascertained 48 h later; no significant difference in either measure was observed. In the latter study, mice that had been exposed to light-duty diesel exhaust (up to 8 h/d, 7 d/wk, for 46 wk) were treated with aerosols of *Streptococcus pyogenes* or *Salmonella typhimurium*; mice that had been exposed to exhaust showed slightly increased toxic responses to streptococci. These results were confirmed in later studies by Campbell *et al.*[17a] showing greater mortality of infected mice exposed to diesel than to gasoline (catalyst-treated) engine exhausts.

In brief, minimal changes are observed in pulmonary function and morphology after exposure to diesel-exhaust particles. Although many morphologic studies have been conducted in animals that have received some individual PAHs intratracheally or otherwise, there is little information on resulting alterations in pulmonary *function*. The morphologic changes that are generally classified as metaplastic are discussed later in this chapter.

NERVOUS SYSTEM

Evaluating the effects of any potential toxin on the development and function of the nervous system experimentally is very difficult. Laurie and colleagues[97,98] set about to determine the effects of chronic diesel-exhaust exposure of neonatal rats on spontaneous locomotor activity and on performance in a bar-pressing task. The neonatal rats were exposed to the exhaust at 6 mg/m^3 for 8-20 h/d for 17-42 d, starting on day 1 or 2 of life. Performance was assessed during weeks 5-16. The activity was depressed both during exposure and in the group tested after exposure, compared with a control group; i.e., they required more extensive training. Because published reports had indicated that the gaseous components lacked any such effect, the authors concluded that the particles or their PAH components were the responsible factors. Laurie and Boyes[97] measured the somatosensory and visual evoked potentials in control rats and rats that had been exposed to diesel exhaust during neonatal life. Although only small abnormalities were noted in the visual evoked potential, significantly longer latencies for all the peaks of somatosensory evoked potential were seen in the exposed rats. Because the latter potentials are in the central nervous system, the authors suggested that diesel-exhaust exposure may lead to failure to develop a normally functioning nervous system. These types of studies have not been conducted with animals exposed to individual PAHs, or to mixtures thereof, so it is not known whether these organic components are responsible for the nervous system lesions.

IMMUNE SYSTEM

The effects of chronic diesel-exhaust exposure of rats on the immune system were assessed by Mauderly *et al.*[110] They placed rats in chambers and exposed them to diesel-exhaust particles for various periods under dynamic conditions and gave them sheep red blood cells intratracheally. The numbers of lymphoid cells that produced IgM antibody to sheep red cells were determined in lymph nodes and spleen 7 d after inoculation with the sheep red cells. Only minimal or no effects on the induction of immunity were observed.

Those studies are of particular interest, in view of the long-known damaging effect of some PAHs on lymphoid tissue. In 1937, Haddow and co-workers[59] reported the systemic toxic effects of PAHs, calling attention particularly to damage to lymphoid tissue. Acute exposure of mice to 3-methylcholanthrene reportedly resulted in damage to the thymus that was followed by thymoma formation, a marked reduction in the weights of the spleen and the mesenteric lymph nodes, and degeneration of bone marrow cells.[184] Newborns appeared particularly sensitive, suffering a wasting disease that culminated in death. This toxic effect has also been noted after administration of 7,12-dimethylbenzanthracene (7,12-DMBA) to rats.[23,128] Repeated administrations of dibenz[ah]anthracene, benz[a]anthracene, or anthracene to mice resulted in an increase in stem cells in lymph glands, a decrease in mature lymphoid cells, and a decrease in spleen weight (only for dibenz[ah]anthracene).[70] In rats, findings were similar after treatment with dibenz[ah]anthracene; the effects with anthracene were much less dramatic.

The total immune response of an organism is an expression of the sum of humoral and cell-mediated effects. Humoral effects derive from the activity of B lymphocytes, which on maturation to plasma cells elaborate immunoglobulins; cell-mediated immunity is expressed by T cells. The effects of chronic administration of benzo[a]pyrene (daily subcutaneous injection for 14 d, for a total of 50-400 mg/kg of body weight) on the humoral immune response were summarized by Dean *et al.*[38] There was a marked decrease in this response. (The noncarcinogen benzo[e]pyrene (BeP) was without effect.) A variety of T-cell responses have been tested for sensitivity to benzo[a]pyrene (BaP) administration.[12,38,39,104] The effects of chronic BaP administration, to a total of 400 mg/kg of body weight, on T-cell function were much less marked than those on B-cell activity. Little effect on the incidence of the B6 tumor in inoculated mice or on the growth of the B16 melanoma after intravenous challenge was observed. Furthermore, the resistance of mice to *Listeria monocytogenes* was unaltered by administration of BaP, although the expulsion of the parasite *Trichinella spiralis* was reduced. BaP administration resulted in myelotoxicity, as determined by in vitro clonal bone-marrow assays. But delayed hypersensitivity reactions in the host were unaffected. In summary, the effects of BaP administration on a variety of T-cell functions were not very significant.

It has long been known that carcinogenic PAHs are immunosuppressive; indeed, this aspect of their action was believed responsible, at least in part, for their ability to cause neoplasia. After treatment of mice with 3-methylcholanthrene (3-MC), dibenz[ah]anthracene, or BaP, a prolonged depression of the immune response to sheep red cells was noted; the noncarcinogens BeP and anthracene were ineffective in this regard.[107,163] The effects of the PAHs have been reviewed by Baldwin,[4] who reported a good correlation between degree of immunosuppression and carcinogenicity.

Although the previously cited work implied a link between the two activities, Dale and Hedges[37] and Stutman[164] definitively dissociated immunosuppression from carcinogenicity. Using guinea pigs, Dale and Hedges concluded that the effects of the PAHs were due to generalized toxicity and were not likely to persist long enough to lead to neoplasia. Stutman produced tumors in mice with very low doses of 3-MC--doses that did not influence the immune status of the animals.

To conclude, some PAHs at high doses can alter the immune status of animals when administered to the point of general toxicity, whereas exhaust and emission have not been shown to do so.

SKIN

The major changes occurring in skin after application of emission or PAHs are associated with neoplasia and are discussed later in this chapter.

KIDNEY

The toxicity of diesel fuel to kidney and other tissues has been described in only one report: a sailor cleaned his hair with diesel fuel and was later hospitalized for renal failure.[6] This acute intoxication also resulted in damage to the liver, the gastrointestinal tract, and the lungs. The information presented does not allow further definition of the toxic components responsible for the pathologic condition.

GLANDS, REPRODUCTION, AND TERATOLOGY

Although individual PAHs have pathologic effects on some glandular tissue, little toxicity has been reported after administration of various kinds of emission. The oral administration of 7,12-DMBA to female mice caused the destruction of small oocytes and reduction in the number of growing and large oocytes.[93] This compound also caused specific destruction of the adrenal cortex in the rat.[13] 3-MC administration resulted in destruction of the primordial oocytes in the mouse.[109] 3-MC or BaP given intraperitoneally produced abnormally shaped sperm indicative of damage to the primary spermatocytes and spermatogonia.[183]

With regard to reproduction and teratology, only few PAHs have been tested. The feeding of BaP to female rats resulted in no abnormalities in their ovarian cycle, ovulation, fertilization, or implantation, and few resorptions were observed in treated pregnant rats.[183] Similar findings have been reported for the mouse.[139]

SHORT-TERM MODEL SYSTEMS FOR DETECTING EFFECTS

Whole-animal experiments for assessing toxicity are often expensive and time-consuming. Therefore, alternative approaches have been developed. A variety of short-term biologic model systems are available for assessing the effects of exhaust, its particulate components, and pyrene analogues. These systems are characterized by the use of multiple end points to measure genotoxicity, the use of both bacterial and mammalian cell lines, the use of end points that can be evaluated in relatively short periods (i.e., 1 d to 6 wk), and the incorporation of an exogenous source of metabolic activation for generating the active PAH metabolites. Each end point in concert with a particular cell system has its own unique strengths and weaknesses. Recognizing this fact, the regulatory agencies have required a battery of short-term tests, to provide a more complete picture of the potential biologic activity of a test chemical. The categories of available short-term tests are presented in Table 4-1, with a partial list of some of the particular tests given in Table 4-2 (see Hollstein *et al*.[74] for details).

Examples of the use of these tests in a short-term battery are presented in Tables 4-3 and 4-4. Table 4-3 demonstrates the guidelines that the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) recommended; tests recommended by the Organisation for Economic Cooperation and Development (OECD) are presented in Table 4-4. In general, these batteries include the evaluation of three or more end points from the following list: toxic effects, mutagenesis, DNA damage and repair, chromosomal alteration, and neoplastic transformation.

TOXIC EFFECTS

Toxicity is usually manifested by such end points as cell death, increase in generation time, decrease in respiration, decrease in rate of macromolecular synthesis, and release of particular cell-bound proteins. Many of these end points have been used in bacteria,[102] protozoa,[75] algae,[172] invertebrates,[172] fish,[148] and mammalian cells.[88] Cell death is also used concomitantly with virtually every assay system, to determine the numbers of cells at risk.

MUTAGENESIS

A mutation is any heritable change in the phenotype of an organism or cell resulting from a change in its DNA. A mutation need not be reflected in a change in function. The phenotypic expression of such a change can be detected in a variety of cellular proteins. Examples of genetic markers that use mutagenesis as the end point are given in Table 4-5.

The bacterial systems are best exemplified by the *Salmonella typhimurium* test strains developed by Ames *et al*.[3] This system measures the reversion rate to histidine prototrophy in five test strains that carry specific frameshift and base-pair substitutions at the *his* locus and a series of mutations at the other loci to make the bacteria more sensitive to chemically induced mutation. The deep rough mutation (*rfa*$^-$), ultraviolet-light sensitivity (*uvr B*$^-$), nitrate reductase deficiency (*chl*$^-$), biotin deficiency (*bio*$^-$), and introduction of R factor plasmids are examples of alterations of these test strains to make them more sensitive to chemically induced mutagenesis. Because reversion to *his* prototrophy is being measured, a battery of strains (three to five) must be tested, to ensure detection of point mutation, frameshift mutation, and intragenic deletion. To circumvent this problem, there have been attempts to standardize a forward-mutation assay with *S. typhimurium*. Forward mutations at both the arabinose-resistance (*ara*^{r})[146,178] and 8-azaguanine-resistance (*8-Az*^{r})[156] genes have been described. These assays have the advantages of detecting virtually all mutagenic events, detecting mutagenesis at more than one genetic locus (probably at least three), and requiring the use of only one test strain.

Mutagenesis testing in mammalian cells has used cell types that range from the rapidly growing, easily handled cell lines, such as CHO[155] and V-79,[26,27] to the more difficult testing of in vivo-derived human lymphocytes.[3] Advantages of the CHO and V-79 cells include high plating and cloning efficiencies, pseudodiploidy, and the ability to monitor mutagenesis at a variety of genetic loci. A disadvantage is that these cells have little or no capacity to metabolize xenobiotics, especially pyrenes. Recent results[54] suggest that hamster-derived cell lines, such as CHO, have limited capacity to remove O6-alkylated guanine; thus, they may be deficient in DNA repair.

The merits and limitations of the three most widely used loci for testing with mammlian cells are presented in Table 4-6. Such end points as resistance to purine analogues, to 5-bromodeoxyuridine (5-BUdR), and to ouabain collectively can detect most of the potential genotoxic effects of PAHs.[77] These end points are now being used simultaneously to limit the possibility of false-negative conclusions. In assays for purine-analogue resistance, mutants lacking the enzyme of the purine salvage pathway, hypoxanthine-guanine phosphoribosyl transferase (HGPRT), are identified by their resistance to toxic analogues, such as 8-azaguanine or 6-thioguanine. In assays for ouabain resistance, mutants are detected by their ability to grow in the presence of the glycoside ouabain. The basis of the latter mutation is an alteration in the receptor for the membranal sodium-potassium ATPase system. In the assay for 5-BUdR resistance, an alteration of the enzyme thymidine kinase is responsible for the mutant phenotype. The altered enzyme is unable to "activate" 5-BUdR by catalyzing its conversion to a deoxyribonucleotide; the latter is required for cell death.

Such cells as C3H10T1/2 and BALB/3T3 have also been used in mutagenesis studies; these cells have easily detectable hydrocarbon-metabolizing activity.[36,92] However, they are hypotetraploid, may not detect some recessive mutations, and may not detect some mutations that are expressed codominantly.[95] These cells express a high degree of contact inhibition and low saturation density and thus can be used in bioassays of neoplastic transformation. Recent studies have suggested that such cells can be used to detect simultaneously the mutagenic and transforming capacities of test chemicals.[35] Primary cell strains and in vivo-derived cells have been used in mutagenesis assays; although they have high PAH-metabolizing capacities and are diploid, the difficulty in growing, handling, and evaluating data from these mixtures of cells is an important disadvantage.[120]

DNA DAMAGE AND REPAIR

Assays for DNA damage and repair have also used both bacterial and mammalian cells. Primary DNA damage in mammalian cells has been measured by such end points as selective toxicity in strains of cells deficient in DNA repair,[144] increase in rate of DNA elution under alkaline conditions,[165] formation of specific pyrene-DNA adducts,[133] increase in rate of unscheduled DNA synthesis,[162] increase in incorporation of specific dyes,[11] and increase in incidence of sister chromatid exchange.[180] DNA repair is a specific response to DNA damage. The covalent interaction of chemicals with DNA provokes an enzymatic repair of the damaged regions of DNA.[142] Repair synthesis can be measured in a variety of ways, but incorporation of radioactive precursors into DNA is the simplest.[29,60] A DNA damage-repair system that shows promise in detecting chemically induced DNA alteration uses the rat hepatocyte.[179] This assay has the advantages of using nondividing cells (normal semiconservative DNA replication is suppressed) and using freshly cultured cells that have high endogenous capacity for carcinogen metabolism or activation. It has recently been shown to be effective in detecting the ability of a variety of chemical carcinogens (including many different PAHs) to damage DNA.[136]

An increase in sister chromatid exchange (SCE) may be one of the best measures of DNA damage in humans. This end point, which involves incorporation of 5-BUdR into DNA during two cycles of replication and making the two chromatids stain differently, so that exchanges of material are scorable, seems to develop as the consequence of presumably long-lived DNA lesions in the S phase of the cell cycle.[126]

The exact mechanism of SCE formation is not understood, although it is well known that the frequency of SCE is increased by exposure of cells to known mutagens in vivo or in vitro.[2,127,161] In fact, a linear correlation between mutations induced at specific loci and SCE has been demonstrated in CHO cells.[20] This assay has been used to monitor the exposure of humans to potentially harmful chemicals[51,94,118] and even to cigarette smoke.[76]

The in vivo techniques for detection of SCE can be applied in two basic ways. One method involves 5-BUdR incorporation into bone-marrow cell DNA by inoculation of solutions or implantations of 5-BUdR pellets directly into animals[2] and exposure of the animals to the chemical under study; this method has been used to detect in vivo DNA damage via such substances as cyclophosphamide,[9] styrene,[31] benzene,[117] urethane,[24] and cigarette smoke.[9] The second method involves the incorporation of 5-BUdR into lymphocyte cultures during mitogen-induced activation in vitro; this has been used in the human studies mentioned above. Good baseline data on the incidence and variation of SCE in humans now exist.[18] SCE has also been shown to persist for several days or even months after chemical exposure and thus can serve as an index of acute or chronic exposure to chemicals.[94,118,161] Comparison of rates of formation of SCE and specific DNA adducts suggests that, for several types of mutagens, induction of SCE does not necessarily result from a single specific DNA lesion.[166]

CHROMOSOMAL ABERRATION

Assays for chromosomal aberration are also used to monitor for the mutagenic activity of test chemicals. These assays detect major rearrangements in the chromosomal or chromatid structure and include such end points as chromosomal or chromatid breaks, chromatid translocation, dicentric chromosomes, ring chromosomes, balanced translocation, and inversion.[46,108] Another test for acutely altered chromosomes is the micronucleus test, in which chromosomal damage leads to fragmentation of chromosomes or malfunction of the spindle apparatus, so that whole chromosomes lag behind the rest and, accordingly, form micronuclei.[81] These techniques can be used with tissues derived either in vitro or in vivo much like those used for analysis of SCE. Generally, agents that induce point mutation also induce chromosomal aberration. In humans, mitogen-activated lymphocytes can be used to monitor for the effects of exposure to physical and chemical agents. Exposure to radiation, to such chemicals as alcohol and vinyl chloride, and to cigarette smoke causes increases in chromosomal aberration.[120] Cytogenic end points of aberration are useful, but one should remember that often chemicals induce very few aberrations at concentrations that permit the end point of gene mutation to be readily observed.[141] In recent comparisons of three cytogenetic tests--

induction of chromosomal aberration, induction of micronuclei, and induction of SCE--the third proved to be the most sensitive in testing with several PAHs.[8]

NEOPLASTIC TRANSFORMATION

Neoplastic transformation has been assayed by a variety of in vitro systems, and it is not possible to review all the pertinent literature here. The reader is directed to the recent reviews of Casto and Carver,[21] Heidelberger,[64] and Mishra et al.[115]

Specific cells that have been used for assay of in vitro chemically induced neoplastic transformation include normal rodent (diploid) cell strains,[42,129] established aneuploid rodent cell lines,[36,49,64,84] cell lines derived from human tumors,[80,138] and cell lines initiated from apparently healthy human tissue.[50,85,113,154] Table 4-7 compares properties of some mammalian-transformation systems. These cell lines share the following properties to some degree: They exhibit density-dependent inhibition of cell division and reach a defined saturation density, do not form colonies on soft agar or agarose, and do not give rise to tumors when inoculated into immunosuppressed syngeneic hosts. After transformation by chemicals, they lose the density-dependent inhibition of cell division and form piled-up, criss-crossed foci; they grow on soft agar or agarose, and they form tumors when inoculated into host animals. In addition, many transformed cells exhibit increased fibrinolytic activity,[154] altered morphology in the scanning electron microscope,[106] specific chromosomal arrangement,[10,134] and specific DNA sequences that can be transfected into normal cells, resulting in formation of the transformed phenotype.[33,152] Although each of these cell systems has been successfully used to ascertain the biologic activity of chemical agents, none appears to be capable of universally detecting all classes of chemical carcinogens, low concentrations of all such agents, and relatively weak biologic activity of some chemicals.

MUTAGENESIS

As just discussed, a number of model systems are available for assessing the mutagenic activity of emission, individual PAHs, and their mixtures. These are in two categories: bacterial systems and mammalian cell-culture systems. The activity of emission and its PAH constituents is discussed below relative to both kinds of model.

BACTERIAL MUTAGENESIS

Particulate matter from city air has been tested for mutagenic activity with the Salmonella/microsome system.[131,167,169] In all cases, a positive response was obtained. Furthermore, many of the samples exhibited direct-acting mutagenic activity, i.e., the addition of activating enzymes present in a liver S-9 fraction was not required for mutagenic activity.[30,132,167,168,171] Wang et al.[177] collected air samples from a residential area at an intersection of two heavily trafficked crossroads in the Buffalo, New York, area. Extraction of the particulate fraction with acetone resulted in a preparation highly mutagenic in Salmonella strains TA 98, TA 100, and TA 1537. These investigators also obtained a positive direct mutagenic response with automobile-exhaust samples from a spark-ignition internal-combustion engine (with leaded gas as the fuel). The mutagenic ingredients appeared to originate in motor oil during the combustion process and were not due to lead. Similar results have been obtained by Pitts et al.[132] with atmospheric particulate extracts from the Los Angeles basin, by Teranishi et al.,[168] by Tokiwa et al.[171] with extracts from several Japanese cities, and by Talcott and Wei[167] and Commoner et al.[30] with extracts from other American cities. Unfortunately, the quantitation of some of these studies may be open to question because of filter artifacts. The disposition of the filter apparatus in relation to sunlight, temperature, etc., is important, because these factors may facilitate chemical reactions involving PAHs and may result in artifactual formation of mutagens. This aspect is discussed in Chapter 3.

Soot makes up 2-15% of the mass of fine particles that are present in urban atmospheres.[105] A number of studies have been conducted to establish its mutagenic potential. Kadin et al.[83] have experimentally generated soot from ingredients with varied sulfur composition --i.e., from pyridine, decalin, and o-xylene or from thiophene, decalin, and o-xylene--and have compared its mutagenicity with that of soot obtained from burned kerosene. Dichloromethane extracts of all the soots were mutagenic in a bacterial assay in which a forward mutation of 8-azaguanine resistance was measured. The soots generated from the sulfur-containing and nitrogen-containing ingredients, as well as soots from kerosene or furnace black, exhibited 10-17% of the mutagenic activity of authentic BaP (on a weight basis).

Emission from spark-ignition combustion and diesel engines has been tested for mutagenic activity in the Salmonella system.[27,79,83,101] It is known that particulate emission from light-duty diesel engines is considerably greater than that from light-duty catalyst-equipped spark-ignition engines--i.e., 0.2-1.0 vs. 0.006-0.02 g/mi.[147] Table 4-8 presents data of Claxton[28] relative to comparative mutagenic activity of emission of diesel and spark-ignition engines, of cigarette-smoke condensate, of coke-oven emission, of roofing-tar emission, and of BaP (positive control). The results are reported in terms of revertants/100 μg of soluble dichloromethane organic compounds; the soluble organic components represent approximately 25% of the total mass of the particles. As is evident from the table, cigarette-smoke condensate, roofing tar, and BaP required metabolic activation by an S-9 fraction, whereas diesel-engine exhaust was directly mutagenic. The other kinds of emission were both directly and indirectly mutagenic. The diesel exhaust exhibited a wide range of mutagenic activity, although the high value is probably peculiar to the particular engine that generated the emission. The activity of BaP is far greater than that of emission.

Naman and Clark[119] have determined the quantity of particles emitted and the mutagenic activity of extracts of the exhausts of several spark-ignition engines that burned gasoline, a 90%/10% ethanol blend, or commercial gasohol. The results are presented in Table 4-9. Although the number of revertants per mile differed for each of the four-cylinder engines, the addition of ethanol clearly reduced the direct mutagenic capacity.

Several investigators have determined the mutagenic activity of respirable coal fly ash,[25,69] which does have mutagenic activity in the Salmonella/microsome assay. Virtually all the emissions yield a mutagenic response in this test system.

Extracts from the various kinds of emission contain a large number of PAHs, among which is BaP.[48,89,176] Extracts of diesel particles have been separated on Sephadex LH-20 into six fractions;[61] the contribution of each to the total mass of the diesel extract obtained from a low-sulfur and high-sulfur fuel is shown in Table 4-10. Furthermore, each of these exhausts was obtained before or after passage through an oxidative catalyst. Fraction 1 contained most of the mass of the extracts from both fuel exhausts. However, fractions 3 and 4 contained most of the mutagenic activity. Fraction 3 from the low-sulfur exhaust contained the bulk of the PAHs, including phenanthrene, methylphenanthrenes, fluoranthene, pyrene, methylpyrenes, benzo[ghi]fluoranthene, benzanthracene (BA), chrysene (or benzo[c]phenanthrene), methyl-BAs, and BeP (or perylene).[61] With the high-sulfur fuel, one found, in addition, the methylbenzothiophenes. It is of interest that the low-sulfur fuel gave an exhaust whose mutagenic activity was increased after passage through a catalyst. The reverse was true for the high-sulfur fuel. Furthermore, fraction 4 from the high-sulfur fuel, before oxidative catalysis, proved the most mutagenic.

The major identified components of emission have been tested for mutagenic activity with the Salmonella forward-mutation assay of Thilly and co-workers.[83,101] In this assay, mutants that are resistant to the purine analogue 8-azaguanine are scored. Of the components present in kerosene-soot extract, cyclopenta[cd]pyrene proved the most mutagenic; it was also present in the highest concentration (see Table 4-11). Cyclopenta[cd]pyrene is a known component of all soots,[53,174,175] of cigarette smoke,[159] of automobile exhaust,[57] and of coal fly ash.[25] The sum of the mutagenicities of the identified individual PAHs was slightly greater than that of the kerosene-soot extract itself. The total mutagenic activity of the kerosene-soot extract could almost be reproduced by that of the cyclopenta[cd]pyrene.

The investigators compared the mutagenic efficacy of additional PAHs with and without an S-9 preparation, using induced cells from the liver; the results are in Table 4-12. Methylation of several of the inactive PAHs, such as anthracene and phenanthrene, resulted in the acquisition of mutagenicity. Preliminary evidence has led the Thilly group to suspect the presence of alkyl-substituted anthracene and phenanthrene in diesel-soot fractions that were mutagenic in bacteria.[101] In this series, the most active compound was perylene, which was followed by cyclopenta[cd]pyrene. The mutagenicity of cyclopenta[cd]pyrene in the Salmonella/microsome assay has been confirmed by Eisenstadt and Gold;[45] metabolic activation by the S-9 fraction was required before this mutagenic property was elicited.

Nitrated PAHs

As mentioned previously, emission from either diesel or spark-ignition engines exhibits considerable direct-acting mutagenic activity in the Salmonella/microsome assay, whereas cigarette-smoke condensates, roofing-tar extracts, and BaP do not. This has led several investigators to study engine exhaust for the presence of direct-acting PAH derivatives that might have been produced by gaseous exhaust components--e.g., nitrogen oxides--or by atmospheric oxidative reactions involving ozone. Various nitropyrenes and other analogues have been assayed for mutagenic activity in the bacterial system.[52,96,111,112,124,130] These substances exhibit potent activity in the Salmonella mutagenesis assay. Indeed, it has been estimated by Gorse (personal communication) that the concentration of nitropyrene alone in diesel particulate extracts could account for 13-24% of the total direct mutagenic activity with TA 98. Tokiwa _et al_.[170] have assayed the mutagenicity of the nitrophenanthrenes, 1-nitropyrene, 3-nitrofluoranthene, and 6-nitrochrysene. Each of the parent PAHs was inactive as a direct mutagen, but 6-nitrochrysene was slightly active, nitrophenanthrene was active, and 1-nitropyrene was most active against TA 98 and TA 100. S-9 was not required for this demonstration of mutagenic activity. Pitts _et al_.[132] reported the direct mutagenic activity of 1-, 3-, and 6-nitrobenzo[a]pyrene in the Salmonella/microsome assay. Perylene, another exhaust constituent that is converted to 3-nitroperylene, demonstrated mutagenesis.[132] In a similar fashion, nitrated derivatives of anthracene, fluoranthene, benz[a]anthracene, benzo[k]fluoranthene, and benzo[ghi]perylene--all of which are present in diesel exhaust--exhibited potent mutagenic activity in the Salmonella assay.[16]

The nitropyrenes have been reported as contaminants of xerographic copiers and toners, which may therefore contribute to the problem of mutagenicity.[103,143] Rosenkranz _et al_.[143] have demonstrated the presence of such a mutagenic activity with various Salmonella test strains; they have traced this property to nitropyrenes that were present as impurities in carbon black. In addition to mononitrated components, they were able to identify the 1,3-, 1,6-, and 1,8-dinitropyrenes, 1,3,6-trinitropyrene, and 1,3,6,8-tetranitropyrene as contaminants. All these derivatives demonstrated direct mutagenic activity (see Table 4-13) with both nitroreductase-positive and -negative variants of Salmonella. The mutagenic property of

1-nitropyrene and 1,3-dinitropyrene depended heavily on the endogenous bacterial nitroreductase activity. Insertion of two nitro groups in the pyrene moiety increased mutagenic activity by a factor of approximately 100, although information is insufficient to extrapolate to other PAHs. The most potent of these derivatives was 1,8-dinitropyrene. It was striking that, of the three dinitro derivatives, two acted independently of endogenous nitroreductase; equal numbers of revertants per nanomole are observed in both TA 98 and TA 98 NR. A similar situation occurred with the trinitropyrenes and tetranitropyrene. The mutagenic activity of 1,8-dinitropyrene is the highest ever recorded in the literature.[112] The presence of the 1,6- and 1,8-dinitropyrenes as predominant mutagenic components in diesel-particle extract has been confirmed by Pederson and Siak,[124] who estimated that 15-20% of the total mutagenic activity of the extract may be contributed by these dinitropyrenes (in addition to as much as 24% contributed by 1-nitropyrene).

Sulfur-Containing PAHs

The presence of sulfur-containing heterocyclic PAHs has been reported in various combustion products, particularly from high-sulfur petroleum products (see Chapter 1). In many heterocyclic structures, one aromatic ring has been replaced by thiophene.[86] It is anticipated that the increase in the use of coal, particularly with high sulfur content, will result in substantial environmental pollution with these ingredients. Thus, it is imperative to have a better understanding of the biologic effects of these sulfur-containing heterocyclic PAHs.

The mutagenicity of several sulfur-containing PAHs has been determined in the Salmonella/microsome assay by Karcher *et al.*,[87] and the results are presented in Table 4-14. Of the isomers listed, benzo[2,3]phenanthro[4,5-bcd]thiophene was the most potent; its ring configuration corresponds to that of BaP, although it is more mutagenic than the latter.

ANIMAL-CELL MUTAGENESIS

A number of animal-cell model systems have been used to ascertain the mutagenic effects of combustion-engine emission, as well as other exhaust. These have been reviewed in previous monographs on PAHs.[174,175] Many of the tests depend on the selection of variants on the basis of resistance to 8-azaguanine, 6-thioguanine, ouabain, or deoxythymidine analogues.

Comparative data on the development of 6-thioguanine resistance in Chinese hamster ovary (CHO) cells have been reported by Casto *et al.*,[22] who used extracts of diesel-exhaust particles and coke-oven emission (see Table 4-15). All extracts yielded the same number of mutant cells, which was comparable with that of the positive control, methyl methanesulfonate, a known direct-acting methylating agent.

Curren et al.[34] have tested the production of ouabain-resistant BALB/c 3T3 cells when the latter were exposed to a variety of agents (see Table 4-16). The spark-ignition-engine extract was considerably more mutagenic in this assay than the diesel extracts. A roofing-tar pot sample and coke-oven emission also exhibited greater mutagenic efficacy. The presence of an activating system did not significantly affect mutagenicity. The extract from the gasoline-engine exhaust appeared more mutagenic than the extracts from various diesel engines, and coke-oven pot samples were even more active. Mutability at several different genetic loci by PAHs has been determined by Huberman and Sachs[78] (Table 4-17). Good mutagenic activity with respect to the HGPRT locus was manifested by dibenz[ac]anthracene, dibenz[ah]anthracene, 7-methylbenzanthracene, BaP, 7,12-DMBA, and 3-MC. The last four compounds named were also mutagenic with respect to ouabain resistance. At both loci, 7,12-DMBA was most active.

As indicated previously, diesel exhaust demonstrates considerable direct mutagenic activity in the Salmonella/microsome assay. The nitro-PAHs have been considered as likely candidates for this activity. Thilly and colleagues[101] have been unable to demonstrate any direct mutagenic activity with human lymphoblasts as the target cells, although, in the presence of an activating system, a considerable amount of 6-thioguanine resistance and trifluorothymidine resistance resulted after addition of diesel extract to the culture media. These experiments suggest that the nitrated PAHs, if present in the diesel extracts, are rapidly inactivated by the lymphoblasts or require for activation a nitroreductase (or other enzyme) that is absent from these cells. Indeed, application of the term "direct-acting mutagen" to the nitrated PAHs is not entirely correct. It is postulated that these analogues undergo a reduction, catalyzed by a nitroreductase, to an amino derivative that may be further transformed into reactive hydroxylamino PAHs (see Chapter 3). The latter would easily form electrophilic substances that could interact with DNA in causing a mutation. What is needed is additional experimentation on the mechanism of action of the nitrated PAHs in both bacterial and mammalian-cell systems.

Sister chromatid exchange has been used to assess genotoxic activity of various kinds of emission. Unfortunately, SCE appears to be more predictive of point mutation than of frameshift mutation,[19] whereas most of the PAHs produce the latter damage. The experiments of Mitchell et al.,[116] which used CHO cells, indicated that all the emission extracts were inferior to BaP in inducing SCE. Of the emissions, coke-oven extracts proved the most active, and the heavy-duty Caterpillar diesel-engine exhaust was the least potent. Intermediate in activity were cigarette-smoke condensate, roofing-tar emission, Mustang gasoline-engine emission, and other diesel-engine emission. None of these required metabolic activation for SCE activity.

The induction of SCE has been performed in vivo with Chinese hamsters that were given various PAHs intraperitoneally.[145] After two injections, the bone marrow was aspirated, and the SCEs per metaphase cell were determined. Although the positive control, BaP, did produce SCE, there was little correlation between the quantitative aspects and the carcinogenic potential of the PAHs. No comparable experiments were performed with the various kinds of emission. The experiments of Schönwald et al.[150] also showed a lack of correlation between carcinogenicity and SCE. These investigators determined SCE induced by BaP with human lymphocytes obtained from normal persons and lung-cancer patients; no difference was observed. Guerrero et al.[58a] intratracheally exposed Syrian hamsters to 200 ng of BaP over a 10-wk period, examined in vitro cultures of lung tissue for sister chromatid exchange (SCE), and concluded from the results that BaP was metabolically activated by lung cells in vivo. In other studies, diesel exhaust particles (DEP) in doses of 0-20 mg per hamster were administered over a 24-h period; although the study was limited in scope, the results demonstrated that DEP can induce genotoxic damage.

CARCINOGENESIS

SKIN

Kotin and colleagues[89-91] first reported the presence of carcinogenic substances in the exhaust of gasoline and diesel engines. Benzene extracts of particles from these sources produced both papillomas and carcinomas when applied to the skin of mice. These studies were extended by Wynder and Hoffmann,[182] who compared the carcinogenicity of cigarette tar with that of organic extracts of gasoline-engine exhaust particles. The latter, obtained from a 1958 gasoline engine without a catalytic converter, proved twice as active (on a weight basis) as cigarette tar. Many studies have since been conducted with skin as the target tissue; only a few are described here.

Automobile-exhaust condensate has been partitioned into a number of fractions by Pott et al.,[135] with the PAHs predominantly found in fraction IV, the nitromethane phase. Each of these fractions was tested[15] for ability to produce papillomas and carcinomas in lifelong mouse skin-painting experiments in which combined initiator and promoter activity was measured. BaP, the positive control, at 1.92-7.68 μg/treatment caused tumor formation in 15-60% of the mice. The exhaust condensate at 0.53-4.7 mg/treatment, equivalent to BaP at 0.15-1.35 μg/treatment, produced tumors in 1-72% of the mice, and the tumors arose after a shorter latent period. The major tumor-producing activity was noted in fraction IV, which contained the PAHs. In this fraction, however, BaP is responsible for only 9% of the carcinogenicity of automobile-exhaust condensate (AEC).[14] Agents other than BaP, acting either alone or synergistically with AEC, are responsible for the major carcinogenicity of AEC and probably of diesel exhaust.

The tumor-producing effects of AEC in the carcinogen mouse model have been contrasted with those of 15 PAHs that occur as major components of AEC.[114] These components and their relative concentrations in a simulated AEC mixture are shown in Table 4-18. AEC, diesel-exhaust condensate (DEC), BaP (the positive control), and the mixture of PAHs were tested for their comparative potency (see Table 4-19). The data indicate the greater potency of AEC than of DEC. If the relative potency of AEC were accepted as 1, the corresponding values for DEC, BaP, and the PAH mixture would be 0.02, 187, and 68, respectively. The proportions of the carcinogenic potency of AEC and DEC attributable to the selected PAHs can be calculated. BaP would account for only 9.6% of this potency in AEC, and the selected PAHs, only 41%. In DEC, the contribution of BaP is approximately 16%. These results indicate that compounds other than the selected PAHs contribute to the carcinogenic potency of AEC or DEC.

Slaga and associates[158] used a mouse that had been bred for quickness of response in the initiation-promotion skin-carcinogenesis model--the SENCAR mouse--to study comparative biologic potency of various kinds of emission and PAHs (see Table 4-20). The exhausts were relatively ineffective, in comparison with purified BaP, in causing papilloma formation. Indeed, 10 mg each of emission from roofing tar, coke ovens, and the Nissan diesel engine was equivalent in response to 50, 60, and 80 g of BaP, respectively. In no case did 10 mg of emission extract contain that much BaP. The activity of anthracene, pyrene, dibenz[ah]anthracene, dibenz[ac]anthracene, benz[a]anthracene, 2-hydroxybenzo[a]pyrene, and BaP as complete carcinogens and as tumor initiators was compared in this mouse strain.[158] Their relative potencies were 0, 0, 20, 0, 5, 30, and 30, respectively, compared with 7,12-DMBA, set at a potency of 100. Schmähl and colleagues extended these studies by determining whether groups of nonactive PAHs would interact with the carcinogens in a synergistic or inhibitory manner.[149] The proportions of the various compounds were chosen on the basis of their relative concentrations in automobile exhaust. The groups of carcinogens and noncarcinogens are shown in Table 4-21, and the percent tumor formation after lifetime application is shown in Table 4-22. Mixtures of the four carcinogens were more effective than a comparable dose of BaP alone. Of greater importance, no evidence of synergism or inhibition could be found when mixtures of carcinogens and noncarcinogens were applied.

The application of multiple PAHs to mouse skin has often resulted in data that were confusing, with regard to carcinogenesis. Thus, in opposition to the above discussion, Steiner[160] reported that the combination of two weak carcinogens, benz[a]anthracene and chrysene, resulted in a synergistic-tumorigenic response; benz[a]anthracene and dibenz[ah]anthracene yielded fewer tumors than expected; and dibenz[ah]anthracene and 3-MC yielded the sum of individual tumorigenic potentials. Falk and co-workers[47] reported much lower tumor production after the simultaneous administration of BaP and several noncarcinogenic hydrocarbons. Van Duuren and Goldschmidt[173] noted that repeated application of the weak carcinogen BeP and the noncarcinogen pyrene to mouse skin with BaP resulted in a cocarcinogenic effect. DiGiovanni *et al.*[41] found that mouse-skin carcinogenesis induced by 7,12-DMBA was inhibited when BeP, pyrene, or fluoranthene was applied 5 min before the initiator. The apparent paradox was explained by the later studies of DiGiovanni and

Slaga.[40] They used either 7,12-DMBA, BaP, or 3-MC as an initiator and tetradecanoyl phorbol acetate (TPA) as the promoter. BeP or dibenz[ac]anthracene was applied 5 min before the initiator in all cases. With 7,12-DMBA as the initiator, BeP and dibenz[ac]anthracene each reduced tumorigenesis by more than 80%. However, with BaP as initiator, dibenz[ac]anthracene exerted no effect and BeP stimulated tumor formation by 30%. If dibenz[ac]anthracene was applied 12, 24, or 36 h before BaP, a reduction in tumorigenesis was observed. With 3-MC as initiator, dibenz[ac]anthracene inhibited tumor formation, whereas BeP was without effect. BeP apparently exerts its effect on the 7,12-DMBA-initiated system by profoundly inhibiting the ring hydroxylation of this initiator and reducing the covalent binding to DNA. Thus, the order of application of the multiple noncarcinogenic with carcinogenic PAHs can have serious effects on carcinogenesis.

Finally, with regard to mouse-skin tumorigenesis, cyclopenta[cd]pyrene, a major component of soot that can transform mouse fibroblasts oncogenically,[122] was tested for tumor-initiating activity on mouse skin by Wood *et al*.[181] Although tumorigenic, cyclopenta[cd]pyrene was weaker than BeP.

TISSUES OTHER THAN SKIN

PAHs and exhaust condensates have been administered to experimental animals in ways other than topically. The subcutaneous injection of AEC and fractions thereof into mice produced sarcomatous lesions;[135] administration of 20-60 mg yielded tumors in up to 8% of mice, and administration of 10 or 90 μg of BaP yielded tumors in 17% or 75% of the animals, respectively. Simultaneous administration of 20 mg of AEC with 90 μg of BaP yielded lower tumorigenesis. The most active fraction from AEC was the nitromethane phase, which contained the various PAHs.

Sellakumar and Shubik[151] studied benz[a]anthracene, benzo[b]fluoranthene, dibenz[ah]anthracene, dibenzo[ai]pyrene, and pyrene. They mixed the PAHs with a hematite dust (at 1:1), suspended the mixture in 0.9% saline, and instilled it intratracheally at weekly intervals into Syrian golden hamsters. Most of the PAHs were not carcinogenic in this limited series, but dibenzo[ai]pyrene produced a high incidence of carcinomas. With multiple doses that totaled 8 mg of this substance, 47% of the hamsters had respiratory tract tumors (squamous cell carcinomas); with 12 mg, 89% of the animals were affected. This degree of carcinogenicity is greater than that of BaP.

Reznik-Schüller and Mohr[137] have compared the carcinogenicity of AEC with that of several major PAH constituents in the Syrian golden hamster intratracheal model. The hamsters were given AEC at 2.5 or 5 mg/animal every 2 wk intratracheally, corresponding to a total administration of 42.5-75 or 75-150 mg of AEC. The total was equivalent to 11.56-25.5 or 25.5-51 μg of BaP. In all animals, multiple pulmonary adenomas were observed. This strikingly high incidence of neoplasia could not be explained by the BaP content of the AEC. It is of interest, however, that no carcinomas were observed.

As indicated earlier and as is discussed more fully in Chapter 6, ingestion of PAHs, whose presence may be attributed to vehicular exhaust, appears to be a major route of entry in animal systems. Yet, the literature pertinent to this form of administration of exhaust particles, their major PAHs, and mixtures thereof is very limited. Neal and Rigdon[121,140] have examined the effects of oral administration of BaP on tumor formation in mice. No gastric tumors developed in any of the 289 mice that were fed a control ration; the incidence of tumors in the BaP-fed mice depended on concentration in the food and on the number of days of feeding.[121] These investigators[140] also established that the incidences of pulmonary adenomas, gastric tumors, and leukemia in BaP-fed mice were genetically determined. No relationship, however, was observed between the relative incidences of these two types of neoplasms within a given mouse. Studies of these types would be useful, with regard to other PAHs and their mixtures. The interpretation of these studies is colored by the failure to house the mice in metabolic chambers, which would eliminate the contribution of coprophagy.

Another series of studies took advantage of the susceptibility of the A strain mouse to pulmonary adenoma formation, particularly after the intravenous administration of selected PAHs.[153] Shimkin and Stoner[153] were able to calculate the amount of each agent that had to be injected for the induction of one pulmonary adenoma in this strain of mouse. The compounds tested were 3-MC, dibenz[ah]anthracene, 7H-dibenzo[cg]carbazoyl, BaP, dibenz[aj]aceanthrylene, and dibenz[ah]-acridine. The respective values were 0.9, 1.0, 6.0, 9.5, 14, and 18 µmol/kg of body weight for one adenoma. Benz[a]anthracene was essentially inactive. The objection to the use of the A strain mouse for these types of studies rests on its extraordinary sensitivity to pulmonary adenoma formation. In fact, if the A strain mouse is allowed to survive long enough, almost all the untreated animals will develop these tumors--they are already "initiated."

ALKYLATED PAHs, MUTAGENESIS, AND CARCINOGENESIS

Because of the presence of alkylated PAHs in cigarette smoke and various coal-derived liquids and tars,[56,62,71,73] their biologic effects are of paramount interest. Perhaps the most thoroughly studied of the alkylated PAHs are the methylbenz[a]anthracenes, methylchrysenes, methylanthracenes, and methylphenanthrenes.

Some of the earliest studies, in which the effects of a methyl group on the carcinogenicity of benz[a]anthracene (BA) were investigated, were conducted by Dunning and Curtiss and by Huggins's laboratory.[82] These investigators monitored sarcoma incidence in rats to which the various PAHs had been administered subcutaneously. Their results, which were in remarkable agreement, indicated that the insertion of a methyl group at position 6 or 7 of BA increased tumorigenicity to the extent that 70-100% of the rats were affected.

However, 8- or 12-methyl-BA resulted in tumor formation in only 50-69% of the rats, and 1-, 2-, 3-, 4-, 5-, 9-, 10-, or 11-methyl-BA proved noncarcinogenic.

The nature of the alkyl group was an important consideration: substitution of an ethyl group at position 7 or 12 of BA greatly diminished tumor incidence, compared with that of the methyl congeners.[123] Pataki and Huggins[123] have also studied the structure-activity relationship in the BA series when two methyl groups were inserted. A marked increase in tumorigenicity--shown by sarcoma formation--was observed with 6,7-dimethylbenz[a]anthracene (DMBA) and 6,8-, 6,12-, 7,8-, 7,12-, and 8,12-DMBA. But, 1,12-, 3,9-, and 9,10-DMBA were essentially nontumorigenic. Of the trimethylated BA derivatives, 6,7,8-, 6,7,12-, and 7,8,12-trimethylbenz[a]anthracenes were all very tumorigenic.

The mutagenicity and tumor-initiating activity of methylated fluorenes, phenanthrenes, anthracenes, and benzofluorenes were studied by LaVoie et al.[99,100] Only the 9-methylfluorene was more mutagenic in the Salmonella/microsome assay than the parent compounds; the 1-, 2-, 3-, and 4-methylfluorenes were as poor mutagens as fluorene itself. In the dimethyl series, 1,9-dimethylfluorene was a potent mutagen in Salmonella TA 100, and the 2,3- and 9,9-dimethylfluorenes were relatively ineffective. Benzo[a]fluorene, benzo[b]fluorene, and benzo[c]fluorene were poor mutagens in the organism, but the 11-methyl derivatives of the first two and the 7-methyl derivative of the latter were more effective. Of this series of methyl derivatives, 11-methylbenzo[b]fluorene was the best mutagen.

In the phenanthrene series,[99] only the 1- and 9-methyl analogues exhibited greater mutagenicity than phenanthrene itself. Equal mutagenic activity was manifested by phenanthrene, the 2-, 3-, and 4-methyl analogues, and the 3,6- and 2,7-dimethyl analogues. The poor mutagenic activity of anthracene was not altered by substitution of a methyl group in position 1, 2, or 9.

Tumor-initiating activity of several of these alkylated PAHs was determined with the mouse-skin two-stage carcinogenesis model.[100] In a series of fluorene, 9-methylfluorene, 1,9-dimethylfluorene, benzo[a]fluorene, benzo[b]fluorene, benzo[c]fluorene, 11-methylbenzo[a]fluorene, 11-methylbenzo[b]fluorene, and 7-methylbenzo[c]fluorene, only 11-methylbenzo[b]fluorene resulted in a marked increase in tumorigenicity. All other compounds exhibited rather weak initiator activity.

The methylchrysenes are known respiratory pollutants that occur in substantial amounts in cigarette smoke--approximately 18 ng/cigarette.[62] Although chrysene itself is generally inactive, several of the methylated species are carcinogenic. In early studies, Gough and Shoppee[55] and Dunlap and Warren[44] showed that the 1-, 4-, and 6-methylchrysenes demonstrated only weak tumorigenicity. The 1,11-dimethyl derivative, however, was moderately active as a skin carcinogen,[32] although less so than 3-MC.

Hecht and colleagues[63] studied a series of methylated chrysenes as both complete carcinogens and initiators. As a complete carcinogen, 5-methylchrysene was far superior to chrysene and the other monomethylated derivatives; it was almost equivalent in carcinogenic potency to BaP. 2-Methylchrysene exhibited about 50% of the carcinogenicity of the 5-methyl analogue. As an initiator, 5-methylchrysene was also the most potent of the methylated derivatives, yielding tumors in 50% of the mice by 14 wk. Next in potency was 3-methylchrysene. The 1-, 4-, and 6-methylchrysenes were all much less effective as tumor initiators. These investigators[63] considered whether 5-methylchrysene, rather than BaP, would be a major contributor to the carcinogenicity of tobacco smoke; but, in view of its small concentration in tobacco smoke, compared with that of BaP (0.6 ng/cigarette vs. 30 ng/cigarette), it is unlikely that this is so.

A series of methylated BaPs were tested for tumor-initiating activity with the mouse-skin carcinogenesis model.[157] Several of the methylated derivatives exhibited greater initiating activity than the parent compound, namely, the 1-, 3-, and 11-methyl analogues. Several were completely ineffective in this regard: the 7-, 8-, 9-, and 10-methyl analogues. The 4-methyl derivative was about equal to BaP in initiating potency.

From these examples, it is apparent that some methylated PAHs are strong carcinogens and therefore should be reckoned with as environmental contaminants.

TOBACCO-SMOKE CARCINOGENESIS

Although the topic has been discussed extensively, several of the potent carcinogenic PAHs that are present in tobacco smoke should be mentioned here.

Approximately half of tobacco smoke consists of particulate constituents in which over 2,000 compounds are represented. The carcinogenicity of cigarette smoke was demonstrated through skin application to the backs of mice and the ears of rabbits[72] and has been confirmed repeatedly in a number of laboratories. Unfortunately, inhalation experiments have not led to as clear-cut a conclusion. When Syrian hamsters were exposed to diluted smoke (smoke-to-air ratio, 1:7) for 10 min twice a day for 18 mo, precancerous lesions were observed in 30%, esophageal tumors in 5%, and laryngeal carcinomas in 10% of the animals; no bronchial or tracheal cancer was seen.[72]

Cigarette-smoke condensates have been partitioned into a number of fractions, of which the most carcinogenic is the "neutral fraction," representing 57% of the mass of the condensate.[72] Although the weakly acidic fraction (approximately 2% of the condensate mass) contained little carcinogenicity itself, 80% of the tumorigenic property of the total condensate could be reproduced in conjunction with the neutral fraction. The neutral fraction was further fractionated by silica-gel chromatography and partitioning between n-hexane and nitromethane into a preparation that contained 0.6% of the total mass, but much of the carcinogenicity. The active nitromethane preparation was further fractionated into two components, each of which contained PAHs. The relative tumor-initiating activity and concentration of several of these ingredients are shown in Table 4-23. BaP, dibenz[ah]-anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, and dibenz[a]-acridine--all present in substantial amounts in cigarette-smoke condensate--are potent carcinogens.

Cocarcinogenicity was demonstrated by applying cigarette-smoke "tar" to the backs of mice in combination with a mixture of 17 major PAHs found in smoke. The concentration of the 17 PAHs was such as not to be tumorigenic. However, the combination of tar and PAHs resulted in tumor formation in 55% of the mice at 13 wk, whereas tar alone yielded tumors in only 18% of the mice. It should be mentioned that various neutral fractions obtained from the cigarette-smoke condensate significantly increased the tumorigenicity of BaP applied topically to mice.

SUMMARY OF ANIMAL-CELL MUTAGENESIS AND CARCINOGENESIS DATA

Although extracts of automobile emission demonstrate mutagenicity in both bacterial and animal-cell systems, their carcinogenicity is not great. Furthermore, attempts to reproduce their pharmacologic activity by assembling mixtures of the major PAH constituents have not been successful. The activity of the major PAHs as carcinogens or mutagens is depicted in summary fashion in Table 4-24. The most potent derivatives in eliciting a mutagenic response in the Salmonella/microsome assay are the nitropyrenes. These have not been tested for carcinogenicity. However, a number of PAHs, such as BaP and some benzofluoranthenes (Table 4-24), are potent carcinogens. There is moderate agreement between the mutagenicity and carcinogenicity of the individual PAHs, although some exceptions are apparent, e.g., fluoranthene. A better predictor of carcinogenicity would consist of a battery of four tests--assays for mutation, chromosomal aberration, primary DNA damage, and morphologic transformation.

TABLE 4-1

Categories of Short-Term Tests[a]

Test Category	No. Methods Identified
Tests in bacteria and phage	13
Tests in eukaryotic microorganisms	19
Mammalian-cell mutagenesis tests	21
In vitro transformation tests	18
Tests of DNA repair and other effects	14
In vivo tests in mammals	14
Tests in insects	4
Mammalian cytogenetics tests	13

---

[a] Data from Hollstein et al.[74] Many assays detect the same genetic event, but are considered separate systems because of other differences, such as in target organism or cell line. Decisions to regard methods sufficiently distinct to be considered separately are arbitrary.

TABLE 4-2

Representative Short-Term Screening Systems

- Bacteria tests:
  - Salmonella/microsome test
- Poly A test (*E. coli*)
- Yeast tests:
  - Mitotic recombination or gene conversion (*Saccharomyces cerevisiae*)
- Mammalian mutagenesis tests:
  - Mouse lymphoma TK +/-
  - CHO/HGPRT
  - Chinese hamster V79
  - BALB 3T3 Oua^R
- Insect test:
  - Drosophila sex-linked recessive-lethal test
- In vivo tests in mammals:
  - Sperm-abnormality test
  - Dominant-lethal test
  - Mouse specific-locus spot test
- Cytogenetics tests:
  - Sister chromatid exchange in vivo
  - Sister chromatid exchange in vitro
  - Chromosomal aberrations in vivo
  - Chromosomal aberrations in vitro
  - Micronucleus test
- Tests of DNA effects and other effects:
  - Unscheduled DNA synthesis (UDS) in human fibroblasts
  - UDS in hepatocyte primary-culture/DNA-repair tests
  - UDS in other target cells
- In vitro transformation tests:
  - Baby hamster kidney cells
  - BALB 3T3 and other cells
  - Hamster embryo cells
  - Enhancement of viral transformation

TABLE 4-3

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Short-Term Battery

For detecting gene mutations, three of the following:

- Bacterial mutagenesis assay, with and without activation
- Eukaryotic microorganisms, with and without activation
- Insects (sex-linked recessive-lethal test)
- Mammalian somatic cells in culture, with and without metabolic activation
- Mouse specific-locus test

For detecting chromosomal aberrations, three of the following:

- In vivo cytogenetics tests in mammals
- Insect tests for heritable chromosomal effects
- Dominant lethal effects in rodents
- Heritable-translocation tests in rodents

For detecting primary DNA damage, two of the following:

- DNA repair in bacteria, with and without activation
- Unscheduled DNA synthesis (repair test) in mammalian cells, with and without activation
- Mitotic recombination and/or gene conversion in yeast cells, with and without activation
- Sister chromatid exchange (SCE) in mammalian cells, with and without activation

TABLE 4-4

Organisation for Economic Co-operation and Development (OECD) Test Guidelines

For detection of gene mutations:

- Salmonella/microsome assay with and without exogenous mammalian (S-9) enzyme activity
- Mammalian-cell point-mutation assay with and without exogenous metabolic activity

For detection of chromosomal damage:

- In vivo cytogenetics assay in rodents
- In vitro cytogenetics assays with and without exogenous metabolic activity:
  - Sister chromatid exchange
  - Chromosomal aberrations

TABLE 4-5

Genetic Markers Developed in Cultured Mammalian Cells

Resistance to cytotoxic chemicals--e.g., 8-azaguanine (8-AG^R), 6-thioguanine (6-TG^R), 5-bromo-2'-deoxyuridine (BUdR^R), α-amanitin, aminopterin, ouabain (Oua^R), cytosine arabinoside, diphtheria toxin

Glutamine or asparagine independence

Auxotrophy (e.g., adenine or proline dependence)

Temperature sensitivity (TS)

TABLE 4-6

Markers for Evaluating Mutagenesis in Cultured Mammalian Cells

Merits	Limitations
Purine-analogue resistance	
Specificity: Low spontaneous background No lethal mutants, because nonessential pathway involved Detects both base-pair and frameshift alterations, with latter more efficient Dominance: X-linked	Cross-feeding occurs; need for refeeding; use of special selection medium
5-Bromodeoxyuridine[a] resistance	
Specificity: No lethal mutants, because nonessential pathway involved Detects both base-pair and frameshift alterations, with latter more efficient May detect chromosomal alterations Expression time: short	Influenced by ploidy; must be heterozygote, because autosomal trait; need for preselection of population before use
Ouabain resistance	
Specificity: low spontaneous background Dominance: independence of ploidy and genotype Artifacts: minimal	Selective responsiveness (reacts only to ouabain mutagens--base substitution); limited spectrum and frequency of mutants; no simple backselection

---

[a]Trifluorothymidine also used.

TABLE 4-7

Comparison of Properties of Some Mammalian Transformation Systems

System	Advantages	Possible Disadvantages
Fischer rat embryo (F1706)	1. Shown to correlate with in vivo test results in double-blind study 2. Easy to discriminate between normal and transformed morphology 3. Transformed cells do not need to be cloned before inoculation into the syngeneic host	1. Not a cloned population 2. Only useful at certain passage levels 3. Low passage cells must be preinfected with a type "C" RNA virus 4. Aneuploid 5. Difficult to quantitate transformation
Hamster in vitro colony assay	1. High levels of mixed-function oxidase activity 2. Diploid chromosome complement 3. Rapidity and reproducibility of test 4. Characterized independently in several laboratories 5. Can be used with metabolic activation 6. Quantitative results possible	1. Low cloning efficiency 2. Heterogeneous population 3. Discrimination between normal and transformed morphologies somewhat subjective 4. Need for pretested, specific lots of fetal calf serum 5. Variation in sensitivity between different embryo pools
BALB/3T3 clone A31 or C3H IOT 1/2 clone B	1. Fairly rapid 2. Easy to discriminate between normal and transformed colonies 3. Well characterized by independent laboratories 4. Can be made quantitative 5. High correlation between phenotypic morphology and tumorigenesis	1. Period of usefulness in terms of sensitivity to focal transformation by chemical carcinogens is unknown 2. Aneuploid 3. Need for pretested, specific lots of serum 4. Intermediate level mixed-function oxidase activity

Table 4-7 (continued)

System	Advantages	Possible Disadvantages
Human cells	1. May reflect more accurately human in vivo conditions 2. Diploid chromosome complement (some systems)	1. Not well characterized 2. Some systems require genetically aberrant target cells 3. Experience necessary to recognize transformed phenotypes 4. Usually a very long latency period 5. Low levels mixed-function oxidase activity

TABLE 4-8

Mutagenic Activity of Various Particulate Emissions and of Cigarette-Smoke Condensates, Compared with Benzo[a]pyrene[a]

Source of Emission	Mutagenic Activity, revertants/ 100 μg of organic material Without S-9	 With S-9
Spark-ignition engine	138	342
Diesel engine[b]	66	Unaltered
	1,225	Unaltered
Cigarette-smoke condensates	0	98
Coke ovens	164	252
Roofing tar	0	99
Benzo[a]pyrene	0	15,202

---

[a] Data from Claxton,[28] who used TA98 strains of Salmonella typhimurium with and without the S-9 activating systems.

[b] Different values were obtained for various diesel engines; the lowest and highest are given here.

TABLE 4-9

Influence of Alcohol on Direct Mutagenicity of Particulate Extracts of Spark-Ignition-Engine Exhaust[a]

Vehicle and Fuel	TA 100 Revertants/ g of Extract	Particulate Emission, mg/mi	Revertants/ mile
Ford Escort:			
Gasoline	10	1.5	15,000
Ethanol blend[b]	9	1.1	9,900
Gasohol	4	1.2	4,800
Oldsmobile Cutlass:			
Gasoline	10	1.7	17,000
Ethanol blend[b]	5	0.6	3,000
Gasohol	13	0.6	7,800
Chevrolet Citation:			
Gasoline	17	1.9	32,300
Ethanol blend[b]	14	0.9	12,600
Gasohol	10	1.0	10,000
Mercury Monarch:			
Gasoline	16	7.1	114,000
Ethanol blend[b]	12	2.8	34,000
Gasohol	20	1.1	44,000

---

[a] Data from Naman and Clark.[119]

[b] 90% gasoline-10% ethanol.

TABLE 4-10

Mutagenic Activity of Sephadex LH-20 Fractions of Extracts of Diesel Exhaust Obtained from Combustion of Low-Sulfur and High-Sulfur Fuel[a]

Fraction	Mutagenic Activity (Revertants/μg)					
	Low-Sulfur Fuel			High-Sulfur Fuel		
	% Mass	With Catalyst	Without Catalyst	% Mass	With Catalyst	Without Catalyst
Total extract		1.0	0.5		0.1	0.5
Fraction 1	70	0	0	59	0	0
Fraction 2	21	0	0	31	0	0
Fraction 3	3	6	8	3	0.6	0.4
Fraction 4	1	8	3	1	5.0	23
Fraction 5	1	3	0.3	1	0.6	0.8
Fraction 6	4	0	0	5	0	0

[a] Data from Hanson *et al.*[61] Diesel exhaust was collected after combustion of 0.25%-sulfur (low-sulfur) or 0.77%-sulfur (high-sulfur) fuel. Catalytic converter used was of monolithic oxidation type. Diesel particles were collected in filters and extracted with dichloromethane--i.e., total extract. Extract was then fractionated by Sephadex LH-20 chromatography.

TABLE 4-11

Bacterial Mutagenic Activity of PAHs in Kerosene-Soot Extract[a]

Compound	Weight % in Extract	Mutation Contribution, induced mutant fraction x 10^5	
		Conc. Extract = 20 μg/ml[b]	Conc. Extract = 100 μg/ml
Cyclopenta[cd]pyrene	15	30	165[c]
Pyrene	8	0	1.7
Benzo[ghi]perylene and anthanthrene	8	2.6	3.4
Coronene	5	0	105
Phenanthrene and anthracene	2	0	0
Perylene	2	1.4	34
Benzo[a]pyrene and benzo[e]pyrene	1	0.6	3.4
Uncharacterized	18.3	--	--
Total CH_2Cl_2	100	20	150

---

[a] Data from Kadin _et_ _al_.[83]

[b] Kadin _et_ _al_. determined the amounts of the individual PAHs in the kerosene soot and, knowing the mutant fractions that these amounts would induce from a dose-response curve, were able to estimate mutagenic contributions that compounds would elicit. The induced mutant fraction = [(no. colonies exhibiting azaguanine resistance in presence of mutagen)/(no. azaguanine-resistant colonies in absence of mutagen)](dilution factor).

[c] Because of nonlinearity of dose-response relationship, compounds may contribute differently to mutagenic response, depending on amount of soot extract and therefore on amount of individual PAH.

TABLE 4-12

Mutagenic Efficacy of PAHs in Relation to Benzo[a]pyrene[a]

Compound	Concentration, µg/ml	Relative Mutagenic Potency[b]
2-Methylanthracene	15.4	0.15
9-Methylanthracene	14.4	0.08
1-Methylphenanthrene	15.4	0.50
2-Methylphenanthrene	7.7	0.30
Pyrene	28.3	0.07
1-Methylpyrene	17.3	0.05
Cyclopenta[cd]pyrene	13.9	1.51
Benz[a]anthracene	14.8	0.14
Chrysene	10.3	0.20
1,2-Benzodibenzo[bd]thiophene	117.0	0
Fluoranthene	1.0	1.00
Benzo[a]pyrene	1.3	1.00
Benzo[e]pyrene	22.7	0.11
Perylene	2.8	6.00
Anthanthrene	12.1	0.08
Dibenz[ac]anthracene	3.6	0.77
Dibenz[ah]anthracene	20.9	0.08
Coronene	51.0	0
or Anthracene	40.0	0
or Phenanthrene	53.4	0
or Dibenzo[bd]thiophene	55.2	0

---

[a] Data from Kadin *et al.*[83]

[b] Relative to benzo[a]pyrene, set at 1.00; rate-limiting factor is concentration that produced too much cell death.

TABLE 4-13

Mutagenicity of Nitrated Pyrenes in *Salmonella typhimurium* TA 98 and TA 98 NR[a]

Compound	TA 98[b]	TA 98 NR[b]	TA 98/TA 98 NR
1-Nitropyrene	484	35	14
1,3-Dinitropyrene	28,600	4,900	5.8
1,6-Dinitropyrene	36,350	37,850	1.0
1,8-Dinitropyrene	75,500	75,500	1.0
1,3,6-Trinitropyrene	31,400	28,220	1.1
1,3,6,8-Tetranitropyrene	7,700	5,200	1.5

---

[a] Data from Mermelstein *et al.*[112]

[b] Strains TA 98 and TA 98 NR are nitroreductase-positive and -negative, respectively.

TABLE 4-14

Mutagenicity of Sulfur-Containing Heterocycles[a]

Material	Dose, µg	No. Revertants (TA 98)[b]	Occurrence
Control	--	36	--
Benzo[a]pyrene	0.5	78	--
	2.0	277	--
Dibenzo[bd]thiophene	20.0	27	Tobacco smoke
Phenanthro[4,5-bcd]-thiophene	0.5 1.0	73 85	Coal tar; carbon black
Benzo[b]naphtho-[2,1-d]thiophene	10.0	44	--
Benzo[2,3]phenanthro-[4,5-bcd]thiophene	0.5	122	Coke-plant effluent
Triphenylene[4,5-bcd]-thiophene	10	42	--
Dinaphtho[2,1-b;1',2'-d]thiophene	10	36	--

---

[a] Data from Karcher *et al*.[87]

[b] In presence of activating S-9.

TABLE 4-15

Mutagenesis in CHO Cells[a]

Addition	Concentration Yielding Comparable Mutation Frequency,[b] µg/ml
Positive control--methyl methanesulfonate	175
Diesel-exhaust particulate extracts[c]	100-275
Spark-ignition engine extract	200
Coke-oven emission	175

---

[a] Results extrapolated from data of Casto _et al_.[22] CHO cells were treated with test agent at various doses for 16-24 h. Cells were collected, and 10^5 cells were inoculated into dishes. Mutant cells were selected for resistance to 6-thioguanine.

[b] Concentration of test agent yielding mutation frequency of 5×10^5.

[c] Extracts obtained from two different engines.

TABLE 4-16

Mutation Frequency of Test Agents with Bal/Bc 3T3 Cells[a]

Source of Emission Extract[b]	Mutation Frequency[c] Without Activation	With Activation
Solvent control	0.18	0.26
Positive control, MNNG (1 μg/ml)	35.5	--
Diesel engine	0.18-1.06[d]	0.20-1.81
Spark-ignition engine	4.49	3.97
Roofing-tar pot sample	3.14	1.73
Coke oven	8.17	--
Benzo[a]pyrene (12.5 μg/ml)	--	14.2

---

[a] Data from Curren et al.[34]

[b] Particles were extracted with dichloromethane. Extract was used at seven concentrations in mutagenesis assay in absence of metabolic activation. Dose ranges included: diesel extract, 10-300 μg/ml; roofing tar, 10-300 μg/ml; spark-ignition engine, 2.5-500 μg/ml; and coke oven, 10-1,000 μg/ml.

[c] Number of ouabain-resistant colonies per million viable exposed cells.

[d] Diesel exhausts from one heavy-duty and two light-duty engines are included; former yielded lower value.

TABLE 4-17

Induction of Ouabain- and 8-Azaguanine-Resistant Mutants by PAHs[a]

Treatment[b]	Cloning Efficiency, %	Mutants/10^6 Survivors Ouabain-Resistant	8-Azaguanine-Resistant
Solvent	92	1	6
Pyrene	94	1	5
Phenanthrene	79	1	8
Chrysene	85	2	9
Benz[a]anthracene	92	2	9
Dibenz[ac]anthracene	95	3	22
Dibenz[ah]anthracene	79	4	17
7-Methylbenz[a]-anthracene	61	24	75
Benzo[a]pyrene (0.3 μg/ml)	27	45	128
7,12-Dimethyl-benzanthracene (0.01 μg/ml)	50	22	41
3-Methylcholanthrene (0.3 μg/ml)	41	38	152

---

[a] Data from Huberman and Sachs.[78]

[b] All compounds added at 1 μg/ml unless otherwise stated.

TABLE 4-18

Weight Proportion of Various PAHs in a Simulated "AEC" Mixture[a]

Component	Weight, µg
Benzo[c]phenanthrene	0.08
Cyclopentenopyrene	1.85
Benz[a]anthracene	0.09
Chrysene	0.21
Benzo[b]fluoranthene	0.17
Benzo[k]fluoranthene	0.06
Benzo[j]fluoranthene	0.09
Benzo[a]pyrene	0.30
1,12-Methylenebenzo[e]pyrene	0.14
10,11-Methylenebenzo[a]pyrene	0.05
Dibenzo[aj]anthracene	0.10
Indeno[1,2,3-cd]pyrene	0.21
Dibenz[ah]anthracene	0.02

---

[a] Data from Misfeld.[114]

TABLE 4-19

Carcinogenic Activity of AEC, DEC, and PAHs on Mouse Skin[a]

Treatment, μg		% Tumors	Latency Period, wk
Solvent control		0	--
Benzo[a]pyrene:	3.85	32.8	74
	7.69	60.9	61
	15.4	89.1	44
AEC:[b]	290	10.3	72
	880	44.3	72
	2,630	83.3	52
DEC:[b]	4,300	0	0
	8,600	2.6	102
	17,150	12.7	76
Mixture of PAHs:[c]			
	3.5	1.3	91
	10.5	38.7	73

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[a] Data from Misfeld.[114]

[b] Obtained with leaded fuel.

[c] See composition in Table 4-18.

TABLE 4-20

Carcinogenic Potency of Various Emissions and PAHs[a]

Substance[b]	Potency, papillomas/mouse-mg
Benzo[a]pyrene	46
Roofing-tar emission	0.2
Coke-oven emission	0.3
Caterpillar diesel exhaust	0
Oldsmobile diesel exhaust	0.1
Nissan diesel exhaust	0.3
Mustang gasoline-engine exhaust	0.1
Cigarette-smoke condensate	0

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[a] Data from Slaga *et al*.[158]

[b] Material was applied to mice once as initiator. TPA (2 μg), twice a week, was used as promoter. Amount of emission condensate that yielded linear response of tumors vs. dose was used.

TABLE 4-21

Mixtures of PAHs and Their Proportions[a]

		Amounts, μg		
Carcinogens		1	2	3
Benzo[a]pyrene		1.0	1.7	3.0
Dibenz[ah]anthracene		0.7	1.2	2.1
Benz[c]anthracene		1.4	2.4	4.2
Benzo[b]fluoranthene		0.9	1.5	2.7
Total		4.0	6.8	12.0
Noncarcinogens	4	5	6	7
Phenanthrene	27.3	81.0	243.0	729.0
Anthracene	8.5	25.5	76.5	229.5
Fluoranthene	10.8	32.4	97.2	291.6
Pyrene	13.8	41.4	124.2	372.6
Chrysene	1.2	3.6	10.8	32.4
Benzo[e]pyrene	0.6	1.8	5.4	16.2
Benzo[ghi]pyrene	3.1	9.3	27.9	83.7
Total	65.3	195.0	585.0	1,755.0
Carcinogens + Noncarcinogens		8	9	10
Carcinogens		4.0	6.8	12.0
Noncarcinogens		65.3	110.5	195.0
Total		69.3	117.3	207.0

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[a] Data from Schmähl *et al*.[149]

TABLE 4-22

Carcinogenicity of PAHs in Combination[a]

Treatment, µg		% Papillomas + Carcinomas
Solvent		0
Benzo[a]pyrene:	1.0	14
	1.7	28
	3.0	56
Carcinogens:	4.0	36
	6.8	68
	12.0	71
Noncarcinogens:	65	1
	195	0
	585	1
	1,755	17
Carcinogens + noncarcinogens:	69	50
	117.3	60
	207.0	70

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[a] Data from Schmähl _et al_.[149]

TABLE 4-23

Tumor-Initiating Activity of Cigarette-Smoke Ingredients[a]

Compound	Relative Tumorigenic Activity[b]	Concentration, ng/cigarette
Benzo[a]pyrene	+++	10-50
5-Methylchrysene	+++	0.6
Dibenz[ah]anthracene	++	40
Benzo[b]fluoranthene	++	30
Benzo[j]fluoranthene	++	60
Dibenz[a]acridine	++	3-10
Indeno[1,2,3-cd]pyrene	+	4
Benz[a]anthracene	+	40-70

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[a] Data from Hoffmann *et al.*[72]

[b] Mouse-skin carcinogenesis.

TABLE 4-24

Summary of Carcinogenicity and Mutagenicity of PAHs in Various Emissions

Compound	Carcinogenic Activity[a]	Relative In Vitro Mutagenic Activity[b] Animal	Bacteria
Anthracene	0	0	0
2- or 9-Methylanthracene	0	+	0
1,2-, 1,3-, 1,4-, or 2,3-Dimethylanthracene	0		
9,10-Dimethylanthracene	0/+		
Phenanthrene	0	0	0
1- or 2-Methylphenanthrene	0	+	+
Fluoranthene	0	+	++
2- or 3-Methylfluoranthene	+		
Pyrene	0	+	0
1- or 2-Methylpyrene	0	+	+
1-Nitropyrene			++
1,3-Dinitropyrene			+++
1,6-Dinitropyrene			+++
1,8-Dinitropyrene			++++
1,3,6-Trinitropyrene			+++
1,3,6,8-Tetranitropyrene			++
Cyclopenta[cd]pyrene	+	++	++
Benz[a]anthracene (BA)	+	+	++
1-, 3-, 4-, 5-, or 11-Methyl-BA	0/+		
2-Methyl-BA	0		
6-, 7-, 8-, 9-, or 12-Methyl-BA	++		+[c]
1,7-, 1,12-, 2,9-, 2,10-, 3,9-, 3,10-, 4,2-, 4,12-, 5,12-, or 8,11-Dimethyl-BA	0		
4,5-, 6,7-, 6,8-, 6,12-, 7,8-, 7,11-, 7,12-, 8,9-, or 8,12-Dimethyl-BA	++	++[d]	+[d]
9,10- or 9,11-Dimethyl-BA	+		
Fluorene	0	0	
9-Methylfluorene	0		+
Acridine	0		
Anthanthrene	+		+
Chrysene	+	+	+
1-Methylchrysene	0/+		
2-, 3-, 4-, or 6-Methylchrysene	+		
5-Methylchrysene	++		
Benzo[b]fluoranthene	++		
Benzo[j]fluoranthene	++		
Benzo[ghi]perylene			+

TABLE 4-24 (contd)

Compound	Carcinogenic Activity[a]	In Vitro Mutagenic Activity[b]	
		Animal	Bacteria
Benzo[k]fluoranthene	++		
Benzo[a]pyrene (B[a]P)	++	++	++
2-, 3-, 4-, 6-, 11-, or 12-Methyl-B[a]P	++		
5-Methyl-B[a]P	+		
8-Methyl-B[a]P	0		
1,2-, 1,3-, 1,4-, 1,6-, 2,3-, 3,6-, 3,12-, or 4,5-Dimethyl-B[a]P	++		
Benzo[e]pyrene	0/+	+	+
Perylene	0	0	++
3-Methylcholanthrene	++	++	++
Indeno[1,2,3-cd]pyrene	+		
Dibenz[ah]anthracene (DBA)	+	+	+
2-, 3-, or 6-Methyl-DBA	+		
7-Methyl-DBA	++		
Coronene	0/+	0	0
Benz[a]acridine	+		
Dibenzo[bd]thiophene			0
Dibenz[ac]anthracene	+	+	+

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[a] 0, no tumors; +, tumors in up to 33% of animals; ++, tumors in over 33% of the animals.

[b] Benzo[a]pyrene mutagenicity set at ++.

[c] 7-Methyl-BA.

[d] 7,12-Dimethyl-BA.

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5

EFFECTIVE BIOLOGIC DOSE

In the class of polycyclic aromatic hydrocarbons (PAHs), there are several chemicals that are environmental pollutants; some are carcinogenic in experimental animals, and some are suggested to be carcinogenic in humans.[160] In the body, they are enzymatically converted to reactive forms that bind extensively and covalently to cellular macromolecules.[63,80,151,182] The covalent binding of reactive metabolites of PAHs to DNA is considered to be an essential first step in PAH induction of neoplasia.[63,80,84,128,151,182] The damaged DNA cannot be fixed and results in a mutation within the cell unless enzymatic repair occurs first. There are many pharmacokinetic and enzymatic processes involved before the formation of reactive metabolites of PAHs, which may ultimately form adducts with DNA. Thus, the concentration to which a person is exposed is probably not a good measure of the biologic dose that causes neoplasia[7,61,149] or other PAH-induced toxicoses (see Chapter 4). This chapter develops the theme that some degree of PAH metabolite-DNA adduct formation in the target tissue can be used as a measure of effective biologic dose. The effective biologic dose of a substance is a reflection of its absorption, distribution, metabolism (activation or detoxification), and excretion. In the case of an alkylating substance, such as a PAH, that dose can be measured directly on the basis of the amount of alkylated DNA, itself a reflection of adduct formation. If the accumulation of adducts in DNA is greater than the capacity of the tissue to repair such lesions accurately and greater than the capacity of the tissue to replicate its DNA, then the presence of adducts will be indicative of the effective biologic dose. The chapter begins with a brief discussion of the pharmacokinetics of PAHs. That is followed by a discussion of the metabolism of selected PAHs. The in vivo formation and disappearance of PAH metabolite-DNA adducts are next reviewed in detail. Finally, there is a discussion of the possibility of using PAH metabolite-DNA adduct content as a measure of effective biologic dose for in vitro mutagenesis, initiation of carcinogenesis, and inhibition of replication and transcription.

PHARMACOKINETICS

Many pharmacokinetic and enzymatic processes are involved before a PAH reaches a target cell and is metabolized to reactive metabolites that interact with DNA and other cellular macromolecules (see Figure 5-1). The oxidative metabolism of PAHs is usually by cytochrome P-450, and the formation of excretable glutathione, glucuronide, and sulfate conjugates results in a very complex metabolic profile. Thus, pharmacokinetic information that would enable one to construct mathematical models of the tissue distribution, metabolism, covalent binding to cellular macromolecules, and excretion of PAHs and metabolites as functions of exposure dose are nonexistent. However, sufficient studies have been done to allow some generalization regarding absorption, tissue distribution, and elimination of PAHs (see Santodonato _et al._,[160] pp. 6-1 through 6-27). Most of these studies have only followed radioactivity in various tissues, urine, and feces after administration of radiolabeled PAHs.

PAHs are readily absorbed after administration by various routes and are then rapidly removed from the blood and distributed into a variety of body tissues. Kotin _et al._[109] examined the radioactivity derived from ^{14}C-labeled benzo[a]pyrene (BaP) in various tissues of rats and mice after intravenous, subcutaneous, and intratracheal administration. The blood concentrations resulting from intravenous injection were hardly detectable after 10 min. Radioactivity was found in stomach, intestine, liver, kidney, lung, spleen, testis, myocardium, urine, and feces. The pattern of distribution was independent of route of administration, except that particularly high lung concentrations followed intratracheal administration. These workers did not examine fat or mammary gland. Other investigators have shown that nonmetabolized BaP,[19] 3-methylcholanthrene (3-MC),[19,40] and dimethylbenz[a]anthracene (DMBA)[19,58] accumulate and persist more in fat and mammary tissue than in other tissues. Some PAHs induce neoplasia in the mammary glands of rats.

Rees _et al._[157] examined the mechanisms by which BaP and other PAHs are absorbed from the gut. Accumulation of BaP in everted sacs of small intestine increased exponentially with incubation-medium concentration. The transport of BaP from the sac tissue to the inside medium was found to be proportional to the concentration in the sac tissue. Thus, if the capacity of other tissues to absorb BaP from extracellular fluid (and blood) is proportional to the concentration of BaP in the fluid, then accumulation in the tissues should also be proportional to intragastric concentration. For example, this relationship was observed in adipose and mammary tissue 18 h after oral administration of BaP. Rees _et al._ postulated a mechanism of physical adsorption onto the intestinal mucosal surface and then passive diffusion into and through the intestinal wall. The proportional nature of the accumulation in the tissue can be accounted for by two phases of adsorption, one unilayer and the other multilayer. Even if tissue accumulation of PAHs is proportionally related to exposure dose, these results should not be overinterpreted. The situation is dynamic; the accumulation is transient, in that PAHs are rapidly metabolized and removed from the body. Rees _et al._ observed that BaP disappeared very rapidly from the thoracic duct lymph. Moreover, PAH metabolite-DNA adduct content in various tissues is not linearly related to exposure dose (as discussed later).

A relevant route of environmental exposure to PAHs is deposition in the lung of particles with PAHs on their surfaces. In general, the degree of retention of PAHs in the lung is a function of the size and composition of the particles carrying them. Several investigators have shown that BaP retention by the lung is higher when it is adsorbed on particulate carbon,[39,85] dust,[164] ferric oxide,[85] aluminum oxide,[85] and talc[145] than when it is not; carbon-particle size affects BaP retention, but the size of particulate ferric oxide or aluminum oxide does not.[85] However, some recent studies have suggested that particulate adsorption of PAHs does not alter retention time in the lung or their distribution to other tissues. Adsorption on ferric oxide did not increase the retention time of BaP in hamster lung after intratracheal instillation.[57] Pylev et al.[155] examined the clearance of intratracheally instilled BaP from the hamster lung; the disposition and clearance from liver, kidney, and blood; and excretion into feces and urine. BaP was instilled alone or adsorbed on asbestos or carbon black. Although these studies were limited in scope, it was found that the disposition of BaP from lung to other tissues, the rate of tissue clearance of BaP, and the pattern of BaP excretion were not altered by the introduction of BaP into the hamster either in free form or bound to particles. Obviously, more studies on rates of clearance from the lung and the later fate of particle-adsorbed PAHs are needed to clarify the effects of particle size and composition. However, it can be concluded that distribution to other tissues occurs after pulmonary exposure to particles on which PAHs are adsorbed.

Elimination of PAHs in animals occurs mainly by excretion of conjugated metabolites into the feces.[4,23,109,161,162] There is some excretion of metabolites into the urine--approximately 10% in the study by Kotin et al.[109] Excretion into bile can be very rapid. For example, 6 h after intravenous injection of [^{3}H]BaP, 60-70% of the tritium appeared in bile or conjugated metabolites.[23] PAH clearance from an animal probably is not limited by metabolic rates or biliary clearance of metabolites, but rather is affected by the persistence of nonmetabolized compound in various tissues (such as fat, skin, and mammary gland) or perhaps by adsorption on particles.

The pharmacokinetics of a PAH will be influenced by prior treatment with chemicals capable of inducing enzyme systems that metabolize it. Schlede et al.[161,162] have shown that pretreatment of rats with unlabeled BaP markedly increased the plasma-disappearance rate of a tritiated dose of the same compound given intravenously; the effect was especially marked during the first 5 min after the intravenous administration of the radiolabeled material, and increased clearance lasted for 6 h. This effect of pretreatment with the compound was paralleled by a lower concentration of [^{3}H]BaP in brain, liver, and fatty tissues; similar but more variable results were observed in lung tissue. These influences of BaP pretreatment on a later intravenous dose of [^{3}H]BaP were also observed when the radiolabeled compound was administered orally. 3-MC and DMBA pretreatment of animals produced comparable effects on the metabolic disposition and tissue content of radiolabeled BaP. Pyrene and anthracene pretreatment had little or no such effect on the in vivo disposition of this compound, nor did phenobarbital. In other studies, the biliary excretion of [^{14}C]BaP was shown to be increased by pretreatment with the unlabeled compound; however, no increase in excretion of the ^{14}C-labeled metabolites of BaP into bile was observed after pretreatment with this compound. These findings suggest that conversion of BaP to its metabolites may be the rate-limiting step in its biliary excretion.

METABOLISM OF PAHs

An organism's processing of xenobiotic chemicals is determined by their physical and chemical characteristics. Figure 5-2 summarizes the possible events leading to carcinogenesis in a cell exposed to a xenobiotic toxic chemical. After uptake, the cell may simply excrete the chemical unchanged, as is the case with some metals and apparently inert materials, such as asbestos. A toxicant may contain functional attachment groups, such as hydroxyl or ketone, that can be conjugated to deactivating moieties like glutathione or glucuronic acid by cytoplasmic transferase. If the toxicant is a PAH or other relatively stable molecule, it will be attacked by the microsomal monooxygenases and form an electrophilic intermediate, which can later be conjugated to a deactivating moiety, detoxified, and excreted.

Once an activated electrophile is formed, it can readily attack nucleophilic sites other than the detoxifying substrates, such as nucleic acids and proteins. The formation of adducts between electrophile metabolites of PAHs and DNA is probably a necessary first step in the initiation of carcinogenesis by PAHs. The in vivo formation of PAH metabolite-DNA adducts is discussed later in this chapter.

These biochemical changes to biologically active intermediates depend on the balance between enzyme systems: those enzymes generating and those detoxifying the intermediates. One of the major enzymes involved in activation is aryl hydrocarbon hydroxylase (AHH). It is found in virtually all eukaryotes (and some prokaryotes), has a wide range of specificities for substrate activity, uses a variety of iron-containing cytosolic pigments as the active sites for chemical oxidation (e.g., cytochrome P-450), and is substrate-inducible.[63,134] Many PAHs are capable of inducing one or more forms of cytochrome P-450. There is some evidence that induction is regulated by one gene or a relatively small number of genes in animal-model systems[110,134] and perhaps even in humans (see Chapter 7). The basis for genetic regulation appears to reside in a balance of inducers and receptors that are activated by PAH metabolites; after binding, translocation to the nucleus, expression of induction-specific RNA, and protein synthesis, the generation of specific cytochrome P-450 is observed.[134] In the murine-model systems, genetically controlled AHH activity is correlated with cancer formation caused by PAHs, such as BaP,[110] 3-MC,[110] dibenz[a]anthracene,[111] and DMBA.[110]

Examples of enzymes that can detoxify these metabolic intermediates are UDP-glucuronosyltransferase, glutathione-*S*-epoxide transferase, aryl sulfatase, and epoxide hydrase. These enzymes catalyze the conjugation of the primary oxidative species formed as a result of AHH activity to forms that are sufficiently polar to be excreted from cells and from the body. Some of the conjugating enzymes are also under a form of genetic control,[142] but their role in PAH carcinogenesis is not completely defined. Epoxide hydrase is one of the enzymes that had been thought to function in a manner that results in the detoxification of PAHs; however, it is now established that, for a variety of PAHs, epoxide hydrase can catalyze the formation of dihydrodiol derivatives of PAHs and that these diols may serve as substrates for monooxygenase activity again--resulting in the formation of diol-epoxides.[63] The diol-epoxides constitute at least one of the ultimate mutagenic and carcinogenic forms of PAHs.

Over the last decade, BaP has been the most exhaustively studied PAH carcinogen and has been the prototype compound in developing the mechanism of action of the cellular monooxygenase and cytoplasmic transferases necessary to activate and detoxify PAH carcinogens. A recent exhaustive summary of BaP metabolism dealt with its activation, carcinogenesis, and role in the regulation of mixed-function oxidases and related enzymes.[63] A composite of metabolic products of BaP is shown in Figure 5-3. BaP has been studied in a large number of in vivo and in vitro systems, as well as in cell-free preparations using homogenates, microsomal fractions, and purified enzymes. BaP may form epoxides at several sites around its ring system, and three epoxides (4,5-,7,8-, and 9,10-) have been identified. Research over the last half-decade has implicated the 7,8-diol (bay region*)[94] as the primary precursor for the second round of activation by mixed-function oxidases, both cytoplasmic and nuclear,[80] that form the highly electrophilic 7,8-diol-9,10-epoxide (Figure 5-4), which opens to form a triol carbonium intermediate. This reactive molecule has been shown to be the major species that binds to nucleic acids via the C-10 position of BaP and to exocyclic amino groups of guanine.

Metabolism of many PAHs other than BaP has also been shown to proceed via diol-epoxides, such as benz[a]anthracene,[174,188] chrysene,[116,189] dibenz[ah]anthracene,[190] 5-methylchrysene,[81] 7-methylbenzanthracene (7-MBA),[35,127] DMBA,[16,46,91,132,179] and 3-MC.[105,179] The ease of formation of carbonium ions by these diol-epoxides parallels the observed biologic activity of the parent chemicals.[115] Metabolic profiles on some PAHs other than BaP are available, and salient features of their metabolism are presented below.

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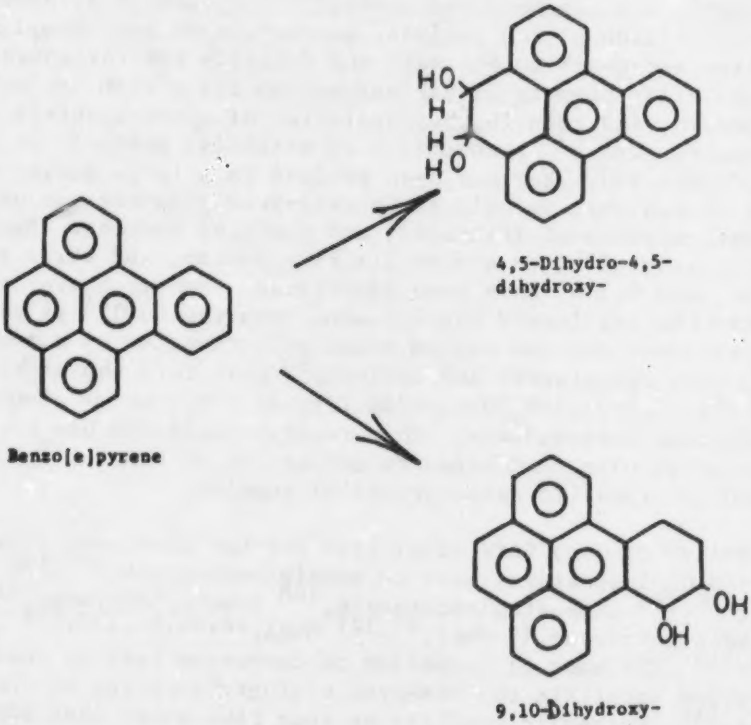
*The bay region is a molecular region between adjacent fused aromatic rings (see reference 115).

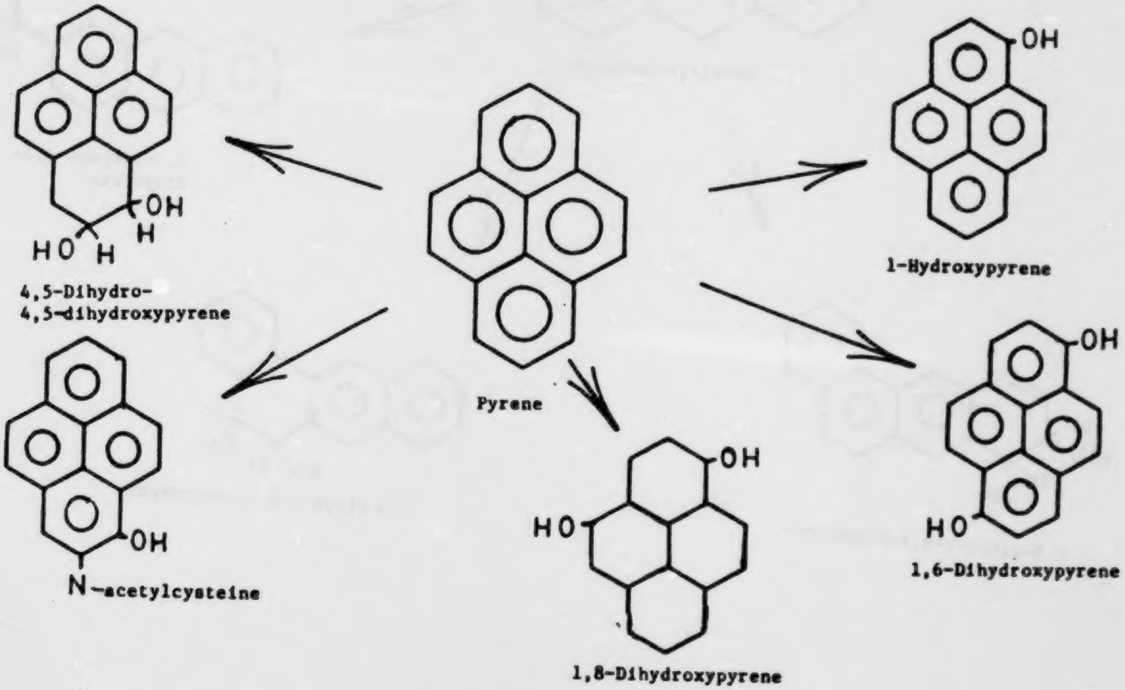
BENZO[e]PYRENE

Benzo[e]pyrene is a marginally carcinogenic structural isomer of the strong environmental carcinogen BaP. It contains two bay regions and, by theoretical calculations, should approximate BaP in carcinogenic activity.[95] Metabolic studies have determined that the probable reason for its lack of carcinogenicity is that its major metabolism is distal to the bay region, so that the molecule does not favor formation of diol-epoxide intermediates. Its metabolism has been studied in hamster embryonic cells and in cell-free preparations from rat liver. Its major metabolite is 4,5-dihydro-4,5-dihydroxybenzo-[e]pyrene. Large-scale experiments with microsomes positively identified 9,10-dihydro-9,10-dihydroxybenzo[e]pyrene, but it constituted less than 1% of the total metabolites.



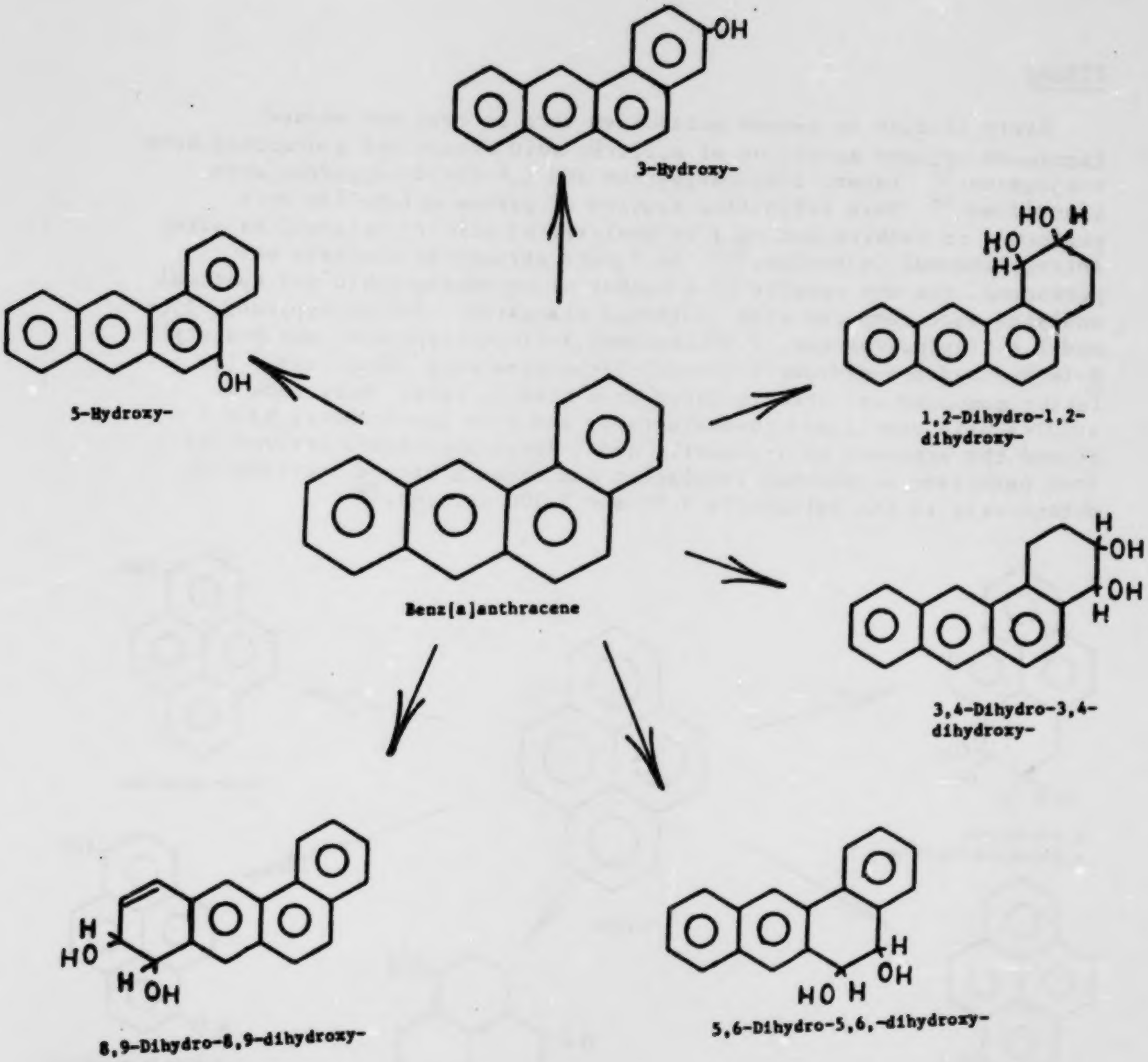
PYRENE

Early studies on pyrene metabolism were in rats and showed increased urinary excretion of sulfuric acid esters and glucuronic acid conjugates.[53] Later, 1-hydroxypyrene and 1,6-dihydroxypyrene were identified.[78] More definitive studies of pyrene metabolism were performed in rabbits and rats by analysis of urinary metabolites after intraperitoneal injection.[172] No direct structural analysis was performed, but the results of a number of chromatographic and spectral analyses were compared with synthetic standards. 1-Hydroxypyrene, 1,6- and 1,8-dihydroxypyrene, 4,5-dihydro-4,5-dihydroxypyrene, and *N*-acetyl-*S*-(4,5-dihydro-4-hydroxy-5-pyrene)-L-cysteine were identified. The latter compound was also isolated from bile in rats. More recent studies with gas-liquid chromatography and mass spectrometry have confirmed the presence of 1-phenolic and 1-dihydroxydihydro derivatives from rat-liver microsomal incubation and shown a marked increase in mutagenesis in the Salmonella T 90 and T 100 strains.[79]



BENZ[a]ANTHRACENE

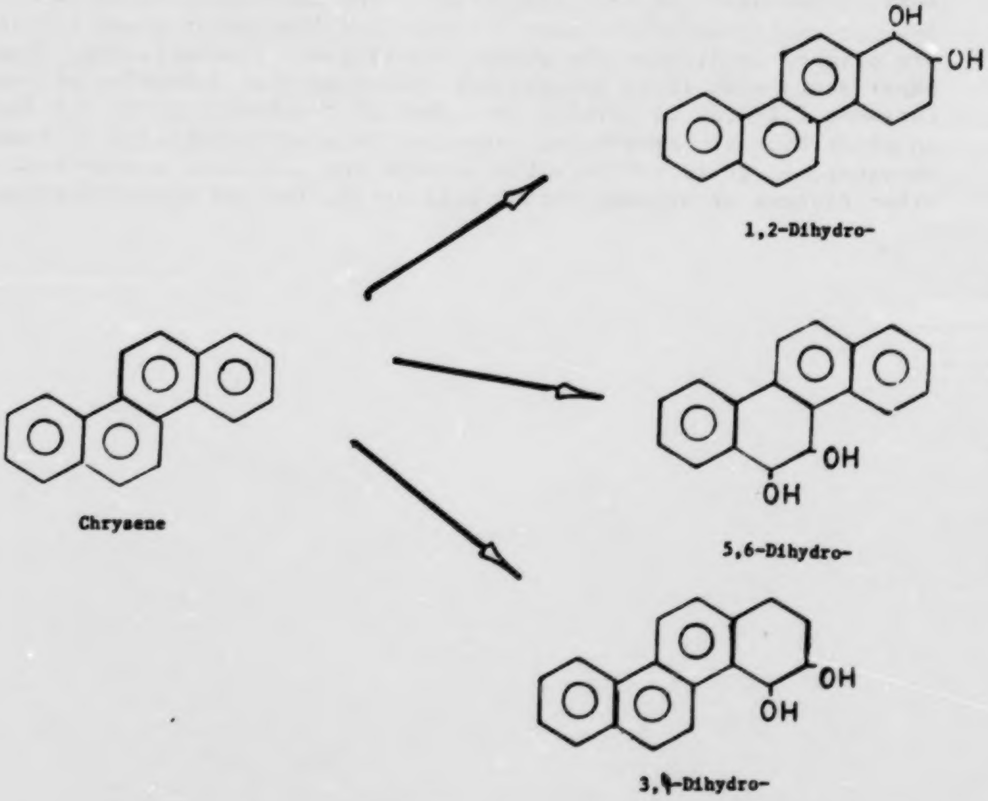
Benz[a]anthracene is a marginally carcinogenic PAH that has both bay-region and K-region areas. The original metabolic studies with benz[a]anthracene were done with thin-layer chromatography.[21,26-28,75,169,171] (See reference 115 for explanation of the K-region.)


3-Hydroxy-
OH
5-Hydroxy-
OH
1,2-Dihydro-1,2-dihydroxy-
HO H
HO
H
Benz[a]anthracene
3,4-Dihydro-3,4-dihydroxy-
H
OH
OH
H
8,9-Dihydro-8,9-dihydroxy-
H
HO
H OH
5,6-Dihydro-5,6,-dihydroxy-
H
OH
HO H

These studies unequivocally identified 5,6-dihydro-5,6-dihydroxybenz[a]anthracene and 8,9-dihydro-8,9-dihydroxybenz[a]anthracene as major metabolites. Also reported were 1,2-dihydrodihydroxybenz[a]anthracene, 3-hydroxybenz[a]anthracene, 4-hydroxybenz[a]anthracene, and benz[a]anthracene 7,12-quinone. In vivo studies[191] with rats, rabbits, and mice reported a mercapturic acid derivative, presumably as a breakdown product of a cysteine conjugate. Also reported were trace amounts of sulfate and glucuronic conjugates at the 3, 4, 8, and 9 positions, presumably as products of phenols; and 10,11-dihydro-10,11-dihydroxybenz[a]anthracene were also reported. The 3,4-dihydro-3,4-dihydroxy derivative of benz[a]anthracene was later confirmed with high-pressure liquid chromatography as a metabolite formed by rat-liver microsomes.[177] This 3,4-dihydrodiol adjacent to a bay region leads to the idea of benz[a]anthracene bay-region activation, including the possibility of an isolated double bond in the 1,2 position after formation of a diol-epoxide. That 3,4-dihydro-3,4-dihyroxybenz[a]anthracene is a minor product quantitatively, as opposed to the less active 5,6-diol, may explain the weak carcinogenicity of benz[a]anthracene.

CHRYSENE (1,2-BENZOPHENANTHRENE)

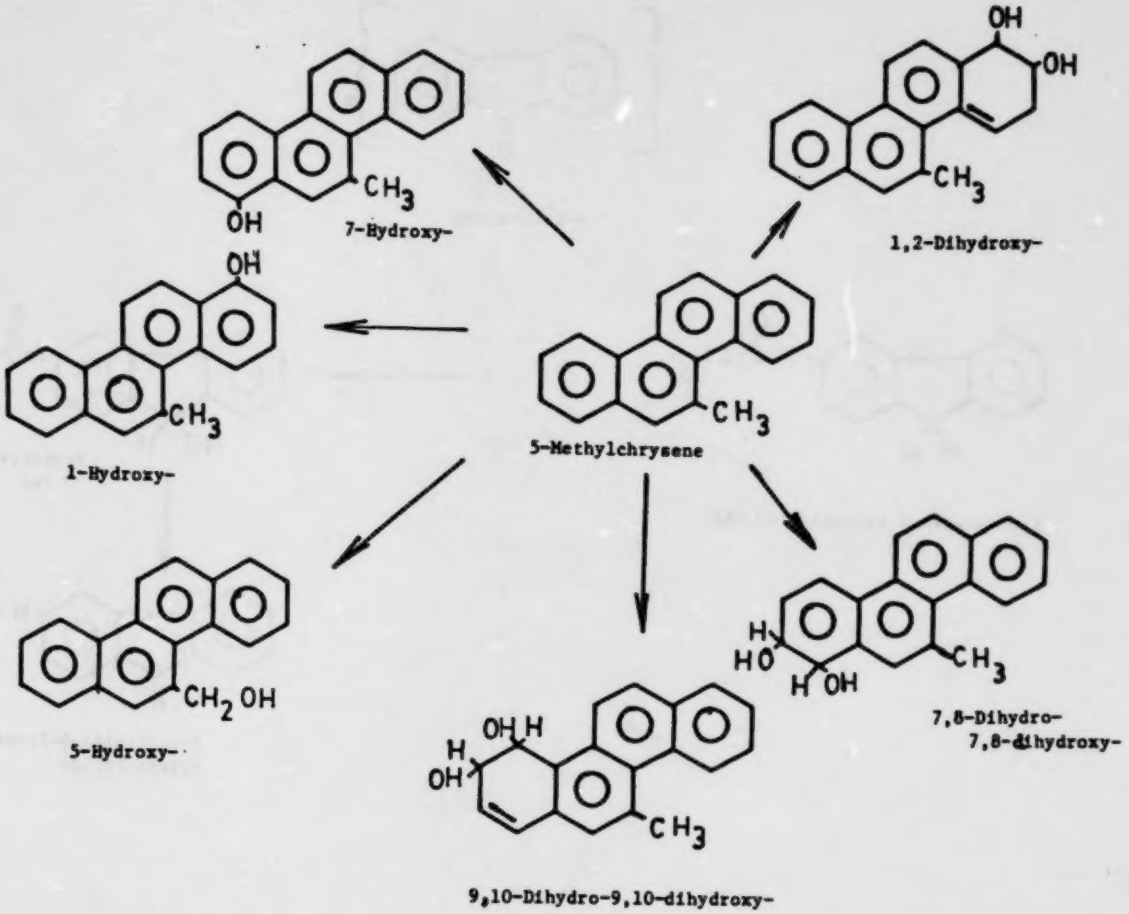
This molecule is composed of two linearly annellated rings formed by pyrocondensation of carbonaceous material and is therefore present in coal tar in substantial quantities.[120] Metabolism of chrysene has been studied in rodents and in cell-free and organ-culture systems. Incubation with rat-liver microsomes produced a series of hydroxylated



metabolites, as seen by high-pressure liquid-chromatographic (HPLC) separation. Several of these metabolites have been identified with the use of synthetic standards.[122] Three dihydrodiols have been characterized: the 1,2-, 3,4-, and 5,6-dihydrodiols. This metabolic profile has been concerned with the use of rat or mouse skin-organ culture.[123] The dihydrodiol metabolites are presumably formed through reactive epoxide intermediates by the P-450 mixed-function oxidases. However, no phenolic or quinoid structures have been identified from the remaining peaks in the HPLC separation.[139]

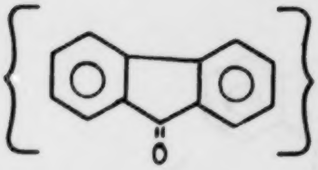
5-METHYLCHRYSENE

Of all the methylchrysenes studied, only 5-methylchrysene shows any substantial carcinogenicity. Metabolism of this compound has been studied in the 9,000-*g* supernatant from rat liver.[81-83] Liver homogenates used for this work were prepared from Aroclor-treated male F-344 rats, and HPLC of metabolites showed nine peaks, of which seven had been identified (according to their relative abundance) as 5-hydroxy-5-methylchrysene, 5-methylchrysene 1,2-diol, 7-hydroxy-5-methylchrysene, 5-methylchrysene 9,10-diol, 9-hydroxy-5-methylchrysene, 1-hydroxy-5-methylchrysene, and 5-methylchrysene 7,8-diol. Two minor metabolites have not been identified. The bay-region theory would predict that 5-methylchrysene 1,2-diol and 5-methylchrysene 7,8-diol are primary candidates for active carcinogenic intermediates. However, experiments with liver homogenates indicated that formation of 5-methylchrysene 1,2-diol is favored over that of 5-methylchrysene 7,8-diol. No other biologic system has been used to study metabolism of 5-methylchrysene, so it is not possible to make any pertinent comparisons with other tissues or between intact-cell activation and detoxification.

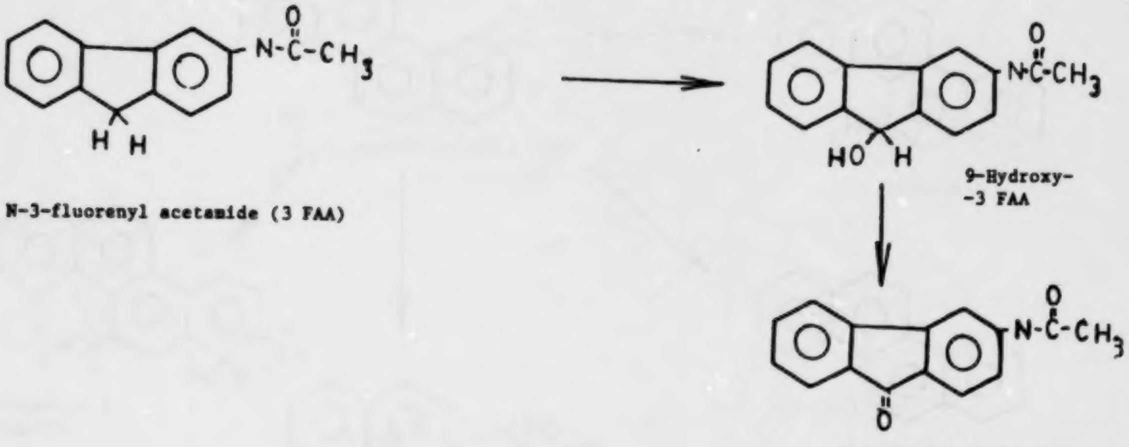

7-Hydroxy-
OH
CH3
OH
OH
1,2-Dihydroxy-
CH3
OH
1-Hydroxy-
CH3
5-Methylchrysene
CH3
CH2OH
5-Hydroxy-
OH H
H
OH
CH3
9,10-Dihydro-9,10-dihydroxy-
H
HO
H OH
CH3
7,8-Dihydro-
7,8-dihydroxy-

FLUORENONE

There appear to have been no direct studies on the metabolism of fluorenone, but N-3-fluorenyl acetamide (3-FAA) yielded two metabolic products.[102] Because the parent compound is carcinogenic, it appears that the derivatives are detoxification products. The authors suggested that metabolism of 3-FAA consists of two sequential reactions: the initial formation of 9-hydroxy-3-FAA as an intermediate to the formation of 3-acetamido-9-fluorene hydroperoxide, which is then dehydrogenated to form 9-oxo-3-FAA. Exposure of rainbow trout to a number of hydrocarbons showed no bioaccumulation of fluorenone; the compound is most likely metabolized to excretible products.[65] However, no attempt was made to analyze any metabolic products. There is no literature on the isolation and identification of fluorenone metabolites.



9-Fluorenone



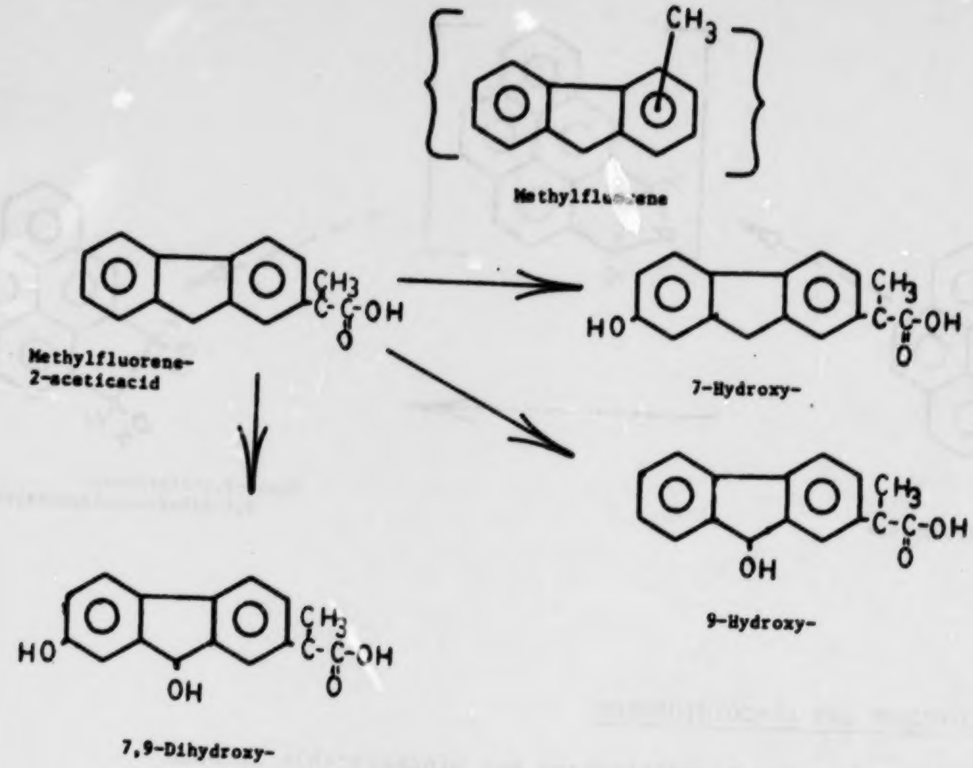
N-3-fluorenyl acetamide (3 FAA)

9-Hydroxy-
-3 FAA

3-Acetamide-9-fluorene-
hydroperoxide

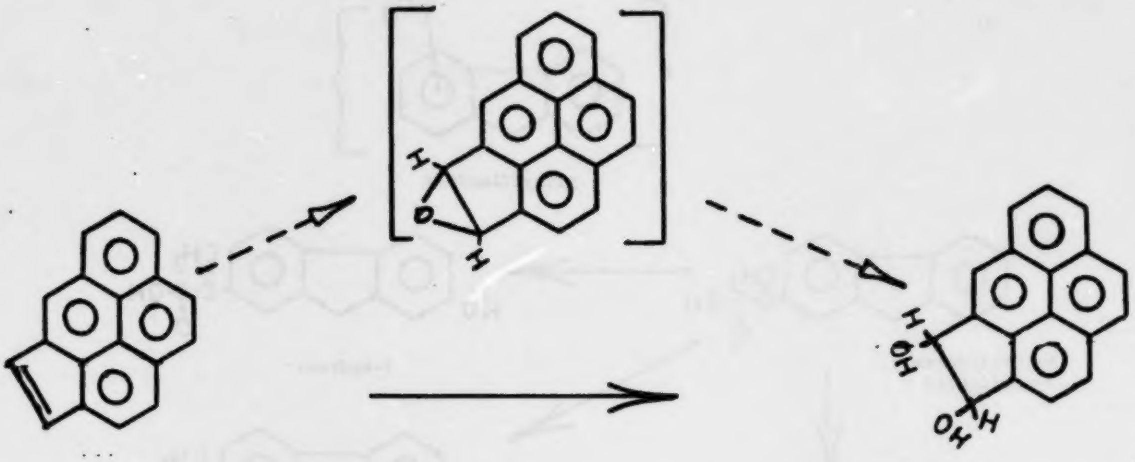
METHYLFLUORENE

There is no literature on the metabolism of methylfluorene, but there has been a major study on methylfluorene-2-acetic acid (MFA) (Cycloprofen, Squibb Institute for Medical Research). This compound is an anti-inflammatory agent whose metabolism has been studied in rats; its metabolites have been isolated from urine and identified. Its major metabolite is substituted at the 7 position on the aromatic ring, so its metabolism may be similar to that of methylfluorene. This congener was given both orally and intraperitoneally. Analysis of the metabolites by thin-layer chromatography yielded six peaks, of which four have been identified. The major metabolite, consisting of 47% of the material, was 7-hydroxy-MFA, with approximately 10% each of 9-hydroxy-MFA and 7,9-dihydroxy-MFA.[42,112]


CH3
Methylfluorene
CH3
C-C-OH
O
Methylfluorene-2-aceticacid
HO
CH3
C-C-OH
O
7-Hydroxy-
CH3
C-C-OH
O
OH
9-Hydroxy-
HO
OH
CH3
C-C-OH
O
7,9-Dihydroxy-

CYCLOPENTA[cd]PYRENE

Cyclopenta[cd]pyrene has been identified as a component of carbon black.[66,69] Its metabolism has been studied in rat-liver microsomes.[67] The major metabolite isolated by HPLC has been identified as trans-3,4-dihydroxy-3,4-dihydrocyclopenta[cd]pyrene. Several components not yet well characterized consisted presumably of phenolic derivatives, as well as metabolites that appear to have saturation of the ethylene bridge.



Cyclopenta[cd]pyrene

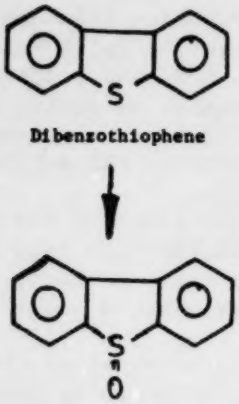
Trans-3,4-dihydroxy-
3,4-dihydrocyclopenta[cd]pyrene

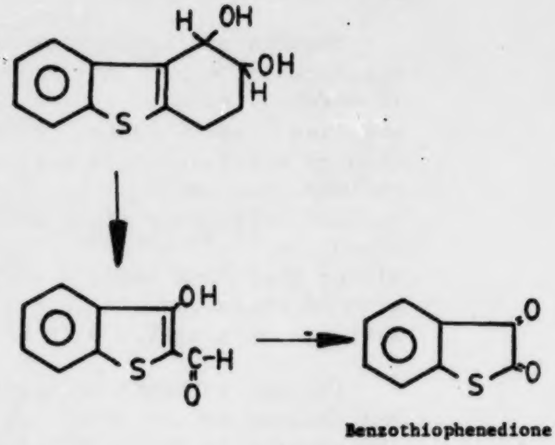
DIBENZOTHIOPHENE AND BENZOTHIOPHENE

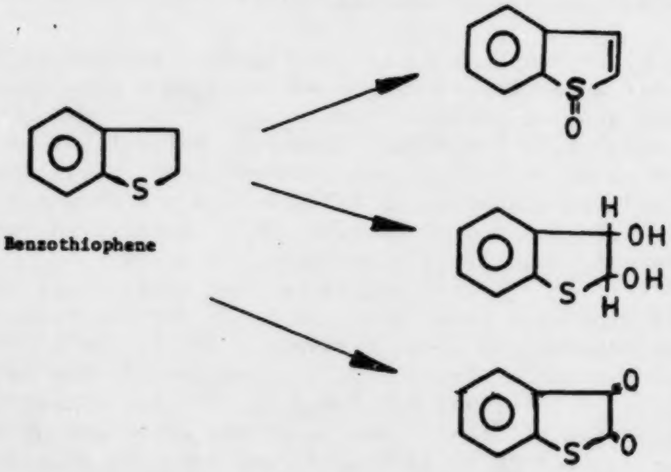
Dibenzothiophene and benzothiophene are biodegradable in both eutrophic and oligotrophic pond waters.[20] Their major metabolite is 1,2-dihydro-1,2-dihydroxydibenzothiophene, with later ring degradation to benzothiophenedione. Benzothiophene and dibenzothiophene form a thioketone, a dihydrodiol (*cis* and *trans* isomers and a diketone). There have been no studies dealing with further metabolism.

Enzymes other than microsomal monooxygenases may also be involved in the metabolic activation of PAHs. Eling *et al*.[52] and Marnett[125] have shown that numerous xenobiotics, including the dihydrodiol metabolites of PAHs, can be cooxygenated during the oxygenation of arachidonic acid by prostaglandin synthetase. In the case of PAHs, when the dihydrodiols are generated, this novel pathway could lead to an alternative pathway for the formation of diol-epoxides. These studies have been done on in vitro model systems. The relevance of their pathway in vivo is unknown.

There is a suggestion in the literature that nitropyrenes are metabolized by bacteria, presumably via a nitroreductase, to produce a high mutagenicity; however, there has been no isolation or characterization of metabolites. Because mammalian cells have much-reduced nitroreductase activity, this rationale has been used to explain the lack of activity in mammalian cells.[62]


Dibenzothiophene


Benzothiophenedione


Benzothiophene

IN VIVO FORMATION AND DISAPPEARANCE OF PAH METABOLITE-DNA ADDUCTS

HISTORICAL PERSPECTIVE

Brookes and co-workers observed that ^{3}H-labeled PAHs applied to the backs of mice[30] or incubated with mouse-embryo cells[48] resulted in covalent binding of radioactivity to DNA, RNA, and protein. Grover and Sims[76] and Gelboin[62] showed that PAHs require metabolic activation by mixed-function oxidases if they are to react covalently with cellular macromolecules. The interactions between PAH metabolites and nucleic acids have since received considerable attention.[63,80,151,182] Identification of the reactive PAH metabolites that form adducts with DNA has been emphasized, because formation of these adducts is believed to be essential for tumor initiation, although interaction with RNA and protein may also be important.

Initial attempts to identify the reactive metabolites that bound to DNA focused on the arene oxide intermediates proposed by Boyland,[25] and especially on the K-region arene oxides, because, according to quantum mechanics, carcinogenic PAHs are distinguished by an electron-rich K region.[10,153] They induce malignant transformation of cells and are active in mutagenicity tests.[84,170] In addition, they are metabolites of PAHs[70,72-74,104,163,170] and will bind to DNA in vitro.[18] However, it became obvious that K-region epoxide-DNA adducts were not the adducts formed in vivo between BaP metabolites and DNA.[18] A similar conclusion was reached in studies with 7-MBA.[17]

Borgen *et al.*[22] found that, in a microsomal activating system, the 7,8-diol BaP metabolite bound to DNA to a much greater extent than any other known diol or phenol. Sims *et al.*[172] provided evidence that the BaP metabolite-DNA adduct formed by BaP metabolism by Syrian hamster-embryo cells in culture was chromatographically identical with an adduct formed by metabolism of BaP 7,8-diol and proposed that the reactive metabolite was a diol-epoxide (DE). Studies in various in vivo model systems, such as cell cultures and organ explants,[13,18,29,71,79,93,172] and in in vivo skin, lung, liver, and forestomach of mice have shown that the major BaP metabolite-DNA adduct observed after exposure of these tissues to BaP is the (+)-BaP DE I-deoxyguanosine adduct. (+)-BaP DE I is apparently the major enzymatic metabolite of (-)-*trans* BaP 7,8-diol.[194] The adduct results from the interaction of (+)-BaP DE I with two amino groups of guanine. The *cis* isomer (-)-BaP DE II is also formed enzymatically from (-)-*trans*-BaP 7,8-diol.[194] The (-)-BaP DE II-deoxyguanosine adduct is formed to the same extent as the (+)-BaP DE I-deoxyguanosine adduct in lung and liver of rabbits, as opposed to the results in mice (C. Bixler and M. W. Anderson, unpublished data). Structures of the BaP DE isomers are shown in Figure 5-5. Although the predominant binding of BaP DE is to the 2-amino group of guanine, these diol-epoxides can also bind to the N7 of guanine,[140] adenine,[98,99,130,175] and cytidine[175] and to phosphate residues.[60,108]

Evidence is accumulating that other PAHs--e.g., 7-MBA,[35,127] benzanthracene,[174,188] chrysene,[116,189] 5-methylchrysene,[81] dibenzanthracene,[190] 3-MC,[105,179] and DMBA[16,46,91,132,179]--are similarly converted to highly reactive diol-epoxides, which then interact with DNA in vivo. All these diol-epoxides have a structural similarity. Jerina and Daly[94] pointed out that the epoxide ring is in a bay region and suggested the term "bay-region diol-epoxides" for these highly reactive metabolites of PAHs.

The bay-region diol-epoxides are mutagenic,[89,96,115,117,126,136,174,185,186] have transforming activity in mammalian cells,[84,126] are carcinogenic in newborn mice[31,100,101,117] and Chinese hamster living cells,[117,187] and are initiators in cells of mouse skin.[31,35,96,100,101,115,117,173] The mutagenicity and carcinogenicity, combined with the observation that bay-region diol-epoxide-DNA adducts are the major adducts formed in vivo in target tissue, have led to the hypothesis that bay-region diol-epoxide adducts are the ultimate carcinogens generated by metabolism of most PAHs.[96,115] However, it should be pointed out that many nonmetabolized PAHs that are termed carcinogenic either lack a bay-region benzene ring or contain nonreactive substitutes in this molecular region.[80] In addition, PAH metabolite-DNA adduct formation in vivo has been examined only for BaP, DMBA, and 3-MC, and these studies have concentrated on target tissues in mice (see Table 5-1).

PAH metabolites other than bay-region diol-epoxides can also bind to DNA. The K-region epoxide of BaP binds DNA covalently.[168,170] Incubation of BaP with microsomes in the presence of exogenous DNA results in a variety of BaP metabolite-DNA adducts.[12,148] In particular, adducts are formed from further metabolism of 9-hydroxy-BaP, possibly the 4,5-epoxy-9-hydroxy-BaP metabolite.[12,106] The major DNA adduct observed after exposure of hepatocytes in culture to BaP resulted from the further metabolism of 9-hydroxy-BaP.[97] A BaP-phenol-oxide-DNA adduct was the major adduct observed in rat lung and liver after intravenous administration of BaP.[23] Various structural modifications of PAH diol-epoxide metabolites do not inhibit binding to DNA.[80,86,87]

Dose-response relationships for formation of PAH metabolite-DNA adducts in target tissue would be helpful in the low-dose extrapolation problem for PAH carcinogenesis.[7,61] Many pharmacokinetic processes determine the extent of formation of PAH metabolite-DNA adducts in an organ after exposure of an animal to a PAH (see Figure 5-1). Although most of these processes have not been completely characterized, some generalizations regarding the extent of adduct formation in vivo can be made from recent reports (see Table 5-1). Previous reviews of covalent binding of PAHs to DNA have not analyzed in vivo adduct formation in detail.[63,80,151,182]

CHARACTERIZATION OF PAH METABOLITE-DNA ADDUCTS

Table 5-1 lists the studies concerned with the in vivo formation of PAH metabolite-DNA adducts. Most of them used mice and BaP.

HPLC analysis of BaP-deoxyribonucleoside adducts formed in lung, liver, and forestomach of A/HeJ mice after oral administration of BaP (3 mg/mouse) is shown in Figure 5-6.[8,184] HPLC analysis is shown for DNA samples isolated by the hydroxylapatite and precipitation procedures. Radioactivity eluted in the water wash (water fraction, WF) and in early portions of the water:methanol gradient that varied from 40% to 70%. This uncharacterized early-eluting radioactivity was much higher in DNA samples isolated by precipitation than by the hydroxylapatite method (see Figure 5-6), although it was still substantial, especially in liver and forestomach, in samples isolated by the hydroxylapatite procedure. Three distinct peaks--I, II, and III in Figure 5-6--were observed in the gradient portion of the chromatography. The specific activity (picomoles per milligram of DNA) associated with these peaks is independent of the procedure used to isolate DNA.[3] Peaks II and III have been identified as (+)-BaP DE I-deoxyguanosine and BaP DE II-deoxyguanosine adducts, respectively.[6] Peak I is probably generated from 9-hydroxy-BaP, although it could be a BaP DE I-deoxycytosine adduct. Small, late-eluting peaks were consistently observed, especially in lung samples (Figure 5-6). They could be BaP DE I-deoxyadenosine or BaP 4,5-oxide adducts.[6] Similar BaP metabolite-DNA adduct profiles were observed in lung, liver, and forestomach from ICR/Ha and C57BL/6J mice after oral administration of BaP.[9,90] Eastman _et al._[51] examined the in vivo binding of BaP to DNA in lung, liver, and kidney of Aroclor 1254-treated A/J mice after intravenous administration of BaP. The only identified adducts observed by Sephadex LH20 chromatography were BaP DE-DNA adducts. Early-eluting radioactivity was present in the chromatograph.[51]

Eastman and Bresnick[50] and Eastman _et al._[51] used Sephadex LH20 chromatography to analyze the 3-MC metabolite-DNA adduct profile in lung and liver of several mouse strains after intravenous administration of 3-MC (Figure 5-7). Two major 3-MC-deoxyribonucleoside adduct peaks were observed in lung and liver of each mouse strain examined. HPLC analysis[49] demonstrated seven 3-MC metabolite-DNA adduct peaks in lung and liver of C57BL/6J mice, with the two major adduct peaks corresponding to those observed by Sephadex LH20 chromatography.[50,51] Early-eluting peaks (Figure 5-7) were also present in the chromatographs of these studies with 3-MC.

Binding of 3-MC, BaP, and DMBA to DNA has been examined in skin of several mouse strains (Table 5-1). In each study with BaP, the major adduct observed was BaP DE-deoxyguanosine. Sephadex LH20 chromatography revealed only one adduct peak. The HPLC adduct profile in skin was virtually identical with that in Figure 5-6 for lung, forestomach, and liver.[11,14,107,149] The adduct profile for 3-MC in mouse skin was the same in each strain examined, but was slightly different from that for lung and liver (Figure 5-7), in that three 3-MC metabolite-deoxyribonucleoside peaks were observed in the Sephadex LH20 chromatograph of skin, whereas only two peaks were observed for lung and liver.[49-51,150] Sephadex LH20 chromatography of DMBA-deoxyribonucleoside adducts in skin was similar in each mouse strain studied; three peaks were observed.[150] Early-eluting peaks were also observed in the chromatographs of these investigations of PAH metabolite-DNA adduct formation in mouse skin.

The formation of DNA adducts of the carcinogen 15,16-dihydro-11-methylcyclopenta[a]phenanthrene-17-one (11-methylketone) was examined in liver, lung, and skin of TO mice.[1,2,38] HPLC analysis revealed eight 11-methylketone metabolite-DNA adduct peaks in each of the tissues.[2] The major adduct was generated from the interaction between the anti-3,4-dihydro-3,4-trans-dihydroxy-1,2-dihydro-1,2-epoxide (diol-epoxide) metabolite of 11-methylketone and deoxyguanosine.[1,2,38] There is no major qualitative difference in the adduct pattern among the three tissues. The adduct profile for each of the three tissues was not substantially altered by the route of administration (intramuscular, topical, and intraperitoneal).

In the studies with mice, the PAH metabolite-DNA binding profiles are very similar in all tissues of all strains examined. For BaP, the predominant characterized adduct is the BaP DE I-deoxyguanosine adduct. When HPLC analysis was used, a BaP DE II-DNA adduct was also observed, as well as an adduct probably generated from 9-hydroxy-BaP. There is also evidence of BaP DE-deoxyadenosine adducts, although in relatively small amounts. The DNA adduct profiles for 3-MC are the same in lung and liver of each mouse strain examined and only slightly different in skin. The pattern of DMBA metabolite-DNA adducts in skin is the same for all strains examined. The HPLC profiles for 11-methylketone metabolite-DNA adducts are very similar in lung, liver, and skin, with the major adduct being a diol-epoxide metabolite-deoxyguanosine adduct.

The in vivo formation of BaP metabolite-DNA adducts has recently been examined in male Sprague-Dawley rats and male New Zealand rabbits (Table 5-1). In rats, BaP was administered intravenously at 1.0 and 10.0 μmol/kg. Several chromatographically distinct nucleoside-bound adducts were observed in lung, whereas only one adduct was apparent in the liver.[23] The predominant BaP-nucleoside adduct formed in vivo in rat lung and liver was chromatographically identical with adducts formed on further metabolism of BaP phenols, possibly because of the interaction of 9-hydroxy-BaP 4,5-oxide with DNA.[6,12,23] The BaP DE adducts were not detected in rat liver, and only a relatively small amount was observed in rat lung. The BaP DE adducts in rat lung accounted for only 1.4% of total DNA binding and 3.3% of the adducts generated by BaP phenol(s). Thus, the in vivo BaP metabolite-DNA adduct profiles obtained in lung and liver of Sprague-Dawley rats are distinctly different from those observed in various mouse strains. This is the only known case in which the BaP DE adduct is not the predominant BaP metabolite-DNA adduct formed in vivo.

In an examination of BaP metabolite-DNA adduct profiles in lung and liver of male New Zealand rabbits, BaP was administered either orally or intraperitoneally at 50 mg/kg. The DNA adduct profiles were identical with those in mice (Figure 5-6), with one notable difference (Bixler and Anderson, unpublished data): In rabbits, there was approximately 75% as much BaP DE II-deoxyguanosine adduct as BaP DE I adduct, whereas in mice, there was only 10% as much BaP DE II adduct as BaP DE I adduct. This is the only known case in vivo in which the BaP DE II-deoxyguanosine adduct approaches the BaP DE I adduct in amount.

It should be emphasized that, in these investigations of PAH metabolite-DNA adduct formation, large amounts of the DNA-associated radioactivity chromatographed not as nucleoside-bound adducts, but rather as uncharacterized fast-eluting peaks (Figures 5-6 and 5-7). Although Adriaenssens et al.[3] showed that isolation of DNA by a hydroxylapatite procedure, instead of by the precipitation method, significantly reduces the amounts of these early-eluting peaks, the peaks still account for a large proportion (especially in liver and forestomach) of the total radioactivity eluted in chromatography. Some workers have ignored these early-eluting peaks by a pre-elution step with Sephadex LH20 chromatography (e.g., Figures 5-6 and 5-7). These peaks are also observed in in vitro studies and in in vivo model systems (see Boroujerdi et al.[23]). The radioactivity appears to reflect some tissue-specific reactions, such as those exhibited by the different patterns in lung and liver.[50,51] Eastman and Bresnick[50] showed, by using borate-eluted Sephadex LH20 and DEAE-Sephadex chromatography, that early-eluting radioactivity contains numerous constituents. Studies that used [^{14}C]BaP and [^{3}H]BaP[5,141,149] and the results of Eastman and Bresnick[50] and Eastman et al.[51] suggested that only a small amount of this radioactivity is due to tritium exchange, whereas experiments of other investigators[15,159] suggested the opposite. The results of Eastman and Bresnick[50] suggested that only a small amount of the radioactivity in the early peaks is related to oligonucleotides. Phosphotriesters might contribute to the early-eluting radioactivity.[50,51] In any case, because a considerable amount of radioactivity appears in the early-eluting peaks, their identification deserves further consideration.

COMPARISON OF EXTENT OF PAH METABOLITE-DNA ADDUCT FORMATION BETWEEN TISSUES AND BETWEEN SPECIES

Specific activities (SAs), in picomoles per milligram of DNA, of PAH metabolite-DNA adducts have been determined in several tissues after administration of PAHs. Table 5-2 gives the SAs of BaP DE adducts in lung and liver of various mouse strains and New Zealand rabbits. The amounts of BaP DE adducts are very similar in lung and liver for each study reported in Table 5-2. Anderson et al.[8] examined the BaP DE adducts in lung, liver, and forestomach of A/HeJ mice for oral administration of BaP at 2-1,350 μmol/kg. The SAs of the BaP DE adducts in lung and liver were similar over the entire dose range and ranged over 3 orders of magnitude in this study. The SA of BaP DE adducts in forestomach of mice is very similar to that in lung and liver after oral administration of BaP.[3,8,90] The similarity of the BaP DE adduct amounts in lung, liver, and forestomach is rather surprising, inasmuch as the disposition and rate of metabolism of BaP in these tissues are probably very dissimilar. The higher rate of BaP metabolism in liver is reflected in the greater total DNA binding and protein binding in liver, compared with lung and forestomach.[8]

The SAs of the BaP phenol-oxide adduct were also very similar in lung and liver of the Sprague-Dawley rat for each intravenous dose of BaP (Table 5-3). As mentioned previously, this was the predominant adduct observed in lung and liver of this species.[23] As with mice, the total DNA binding was significantly higher in liver.

Eastman and Bresnick[50] examined 3-MC metabolite-DNA adduct formation (Figure 5-7) in lung and liver of several mouse strains at several points after intravenous injection of 3-MC (12.6 mg/mouse). Mixed-function oxidases were induced with Aroclor 1254 24 h before injection of 3-MC. The amounts of adducts were significantly higher in lung than in liver in each mouse strain and at each time (Table 5-4). Total DNA-associated radioactivity in liver is not significantly different from that observed in lung. Thus, the relative binding of 3-MC to DNA of lung and liver of Aroclor 1254-treated mice is distinctly different from that of BaP in untreated mice. The ratio of 3-MC-DNA binding in liver to that in lung is smaller than the ratio for BaP for both nucleoside-bound adducts and total DNA-associated radioactivity (Tables 5-3 and 5-4). At present, BaP and 3-MC are the only PAHs for which the amounts of PAH metabolite-DNA adducts can be compared between lung and liver.

It should be emphasized that the SAs for the in vivo studies reported in Table 5-1 are calculated on the basis of the total DNA in the organ. These values for the BaP DE adducts (Table 5-2) do not differentiate between lung and liver and therefore do not appear to offer any explanation for susceptibility of the lung and resistance of the liver to BaP-induced neoplasia in, for example, A/HeJ and A/J mice. However, it is likely that the amounts of adducts formed in different cell types vary considerably. This possibility has the greatest implication for organs, such as the lung, that contain a multitude of cell types. Although little is known about the localization of carcinogen-DNA adducts in lung, cytochrome P-450-dependent monooxygenase enzymes appear to be much more localized in lung than in liver.[43,165,166] The nonciliated bronchiolar epithelial (Clara) cells of rabbit lung have been identified as having high concentrations of these enzymes--a finding that correlates with the observed pulmonary toxicity of 4-ipomeanol, which is thought to result from high covalent binding of a reactive metabolite to proteins in the Clara cells of a number of species.[24] The Clara cells amount to only 1% of the pulmonary cells.[59] The high concentration of cytochrome P-450 in Clara cells may also be important in nitrosamine-induced pulmonary carcinogenesis.[158] Because the average SAs of BaP DE-DNA adducts are similar in lung and liver, the above considerations suggest that adduct contents might be much higher in some pulmonary cell types than in hepatocytes. Also, the removal rates of the BaP DE-DNA adducts might vary considerably in different cell types in the lung. Examination of adducts in individual cell types might allow differentiation of tissues with respect to susceptibility and resistance to PAH-induced neoplasia.

Results of several studies allow comparisons to be made between amounts of PAH metabolite-DNA adducts in the same tissue from different species (strains). BaP DE adducts and 3-MC metabolite-nucleoside adducts (Figure 5-7) can be compared in lung and liver of different strains of mice under the same dosage regimen (Tables 5-2 and 5-4). The BaP DE adduct amounts are very similar in lung of A/HeJ and C57BL/6J mice and 10 times smaller in ICR/Ha mice (Table 5-2). A/HeJ mice exhibit high susceptibility, ICR/Ha moderate susceptibility, and C57BL/6J high resistance to BaP-induced pulmonary neoplasia. Obviously, the SAs of the BaP DE adducts do not differentiate between species in susceptibility to BaP-induced neoplasia. Also, the disappearance rates of BaP DE adducts are similar in C57BL/6J and A/HeJ mice.[9] In contrast, Eastman and Bresnick[50] claimed that their results regarding 3-MC metabolite-nucleoside adducts formed and their disappearance rates do differentiate between 3-MC-induced pulmonary neoplasia in the various mice strains (Table 5-4). It is hard to understand why adduct contents or their disappearance rates based on total organ DNA would differentiate between mouse strains on the basis of pulmonary susceptibility to one PAH and not another. Again, examination of SAs in individual pulmonary cell types might unravel this dilemma.

Phillips _et al_.[150] examined the covalent binding of DMBA, 3-MC, and BaP to DNA in the skin of mice of various strains. Neither the amounts of PAH metabolite-DNA adducts nor their disappearance rates showed a correlation with the reported susceptibilities of the strains to PAH-induced skin carcinogenesis. Thus, the results of this study based on average cellular SAs of the organ are in agreement with the BaP, but not the 3-MC, study of pulmonary adducts in the different mouse strains.[9,50,90] Ashurst and Cohen[11] confirmed the results of Phillips _et al_.[150] with HPLC analysis. Baer-Dubowska and Alexandrov[14] examined the binding of BaP to skin of rats and mice under conditions known to initiate tumorigenesis in the skin of mice. The patterns of BaP metabolite-DNA adduct profiles were identical in the two species and very similar to those in Figure 5-6. The amounts of the BaP DE-deoxyguanosine adducts and total DNA-associated radioactivity were 3 times higher in mouse skin. The adduct difference probably does not differentiate between the mouse susceptibility and rat resistance to BaP-initiated skin tumorigenesis.

DOSE-RESPONSE RELATIONSHIPS OF PAH METABOLITE-DNA ADDUCTS

Although there have been a considerable number of in vivo studies on PAH metabolite-DNA adduct formation (Table 5-1), there are only three reported dose-response studies of amounts of PAH metabolite-DNA adducts.

Phillips et al.[150] treated C57BL mice topically with DMBA at 0.025-1.0 μmol/mouse. DMBA metabolite-DNA adducts in skin were determined by Sephadex LH20 chromatography. Three DMBA metabolite-deoxynucleoside adduct peaks were present at each dose, and the SAs plotted in Figure 5-8 are the sums of these three peaks.

Pereira et al.[149] examined the formation of epidermal BaP-DNA adducts in ICR/Ha mice at topically applied doses of 0.01-300 mg/mouse. The SAs in Figure 5-9 are essentially linear with dose throughout the dose range; the log-log plots have slopes of approximately 1. Peak I in Figure 5-9 represents early-eluting peaks, and peak III in Figure 5-9 represents the BaP DE-deoxyguanosine adducts.

Anderson et al.[8] investigated the dose dependence of BaP metabolite-DNA adducts in the lung, liver, and forestomach of A/HeJ mice (Figure 5-10). BaP was administered orally at 0.048-29.7 μmol/mouse, and animals were sacrificed 48 h later. The SAs plotted in Figure 5-10 are for the BaP DE-deoxyguanosine adducts. Similar dose-response curves are obtained for the early-eluting peaks (WF and IP in Figure 5-6) and the BaP-phenol-oxide adduct (Peak I in Figure 5-6). The curves in Figure 5-10 are either linear with a slope greater than 1 and concave downward or linear with a slope of 1 and concave upward (Figures 5-8 and 5-9, respectively). This means that the percentage of the dose that becomes bound to DNA as BaP DE adducts decreases as the dose decreases in the tissues of the A/HeJ mice (Figure 5-10), whereas the percentage of the dose that becomes bound to epidermal DNA is constant or actually increases as dose decreases (Figures 5-5 and 5-6, respectively). However, the values of the BaP DE adducts in lung and forestomach of A/HeJ mice at the lowest dose examined are only approximately 50% lower than those predicted by simple proportion from the adduct values at the highest dose (Figure 5-10). Thus, the results of these dose-response studies do not reveal the existence of any threshold dose below which binding of PAH metabolites to DNA does not occur.

EFFECT OF AHH INDUCERS ON PAH METABOLITE-DNA ADDUCT FORMATION

The effect of AHH inducers on the in vivo binding of BaP to DNA has been examined in several tissues of various mouse strains.[36,90,184] In a study by Wilson et al.,[184] adducts were determined under conditions known to result in inhibition of BaP-induced pulmonary neoplasia by β-NF. Female A/HeJ mice were fed either a β-NF diet (0.3% of diet) or a control diet for 16 d and then given BaP (6 mg/mouse). The animals were returned to a control diet for 2 wk and then placed on either a β-NF diet or a control diet for another 16 d. This regimen results in a 94% reduction in BaP-induced pulmonary adenomas.[181] In the adduct study, [^{3}H]BaP (6 mg/mouse) was administered to one group (A) of animals, both control and β-NF-treated, on the sixteenth day of the first NF feeding.[184] Another group (B) of mice, control and β-NF-treated, were given unlabeled BaP (6 mg/mouse) on the sixteenth day of the first β-NF feeding and [^{3}H]BaP (6 mg/mouse) on the sixteenth day of the second β-NF feeding. Animals in both groups were returned to control diets after administration of [^{3}H]BaP and sacrificed 48 h later. Table 5-5 gives the percentage decrease in BaP DE-DNA adduct formation in the lung and liver of β-NF-treated mice. The decrease in the amount of BaP DE-DNA adducts in the lung, 86-93%, appears to correlate with the inhibition of pulmonary adenoma formation (94%).[181,184] No BaP DE-DNA adducts were detected in the liver of β-NF-treated mice. Wilson et al.[184] also examined the effects of two other AHH inducers, TCDD and Aroclor 1254, on BaP-DNA adduct formation in lung and liver of A/HeJ mice. These inducers, like β-NF, markedly decreased the formation of the BaP DE adducts in lung and liver (Table 5-5). It is interesting that TCDD and Aroclor 1254 had little effect on or actually increased total DNA-associated radioactivity in the early-eluting peaks. In a similar study, Ioannou et al.[90] investigated the effect of β-NF treatment on BaP DE adduct formation in ICR/Ha mice. β-NF treatment also resulted in a significant reduction, 80-90%, in BaP DE adducts in lung, liver, and forestomach of this strain.

Cohen et al.[36] examined the effect of TCDD treatment on BaP DE-DNA adduct formation and BaP-induced tumor initiation in the skin of SENCAR and CD-1 mice. BaP DE adduct formation in skin was completely inhibited in both strains of mice, and BaP-initiated papilloma formation was inhibited by 93%.[36] Total DNA-associated radioactivity and covalent binding of BaP to skin protein were increased (Table 5-6). The increase in total DNA-associated radioactivity was due to the increase in the amounts of the early-eluting peaks.[36] Again, inhibition of BaP-induced tumor formation correlates with inhibition of BaP DE adduct formation in the target tissue, but not with total DNA-associated radioactivity on covalent binding of BaP to protein. In a continuation of their studies on the anticarcinogenic effects of TCDD, DiGiovanni et al.[44] showed that the time course for induction of epidermal AHH and UDPGT correlated with the time course for the inhibitory effects of TCDD on tumor initiation with DMBA, BaP, and 3-MC.

Thus, AHH inducers inhibit in vivo BaP DE-DNA adduct formation in every tissue of every mouse strain examined. The effects of AHH inducers on BaP DE-DNA adduct formation in vivo contrast markedly with their effects in vitro, i.e., in lung and liver microsomes, isolated peripheral lung and liver tissue slices, and hepatocytes. Treatment of animals with AHH inducers stimulates the formation of BaP DE adducts in vitro (see Wilson et al.[184] for references). The effect of AHH inducers on total DNA-associated radioactivity and on covalent binding of BaP to protein is similar in vivo and in vitro. The reason for the disparity between the in vivo and in vitro results for BaP DE adduct formation is unclear--in particular, the relationship between induction of AHH and decreased in vivo BaP DE adduct formation.

Induction of AHH has been postulated as a mechanism for the anticarcinogenic action of a wide variety of compounds.[36,44,45,90,154,180,181,184] The results of Wilson et al.[184] and Cohen et al.[36] discussed above suggest that AHH inducers, such as β-NF and TCDD, inhibit BaP-induced neoplasia by reducing the amount of BaP DE-DNA adducts formed in the target tissue. The data of Pyerin et al.[154] also suggest that prior AHH induction is a protective mechanism against DMBA-induced tumor initiation in mouse skin. The concept of a protective effect of prior treatment with AHH inducers against PAH-induced tumor initiation might appear to be incompatible with the suggestion that AHH inducibility and PAH-induced tumor initiation may be causally related.[64,103,133,135,143,147,178] Relationships between AHH inducibility and PAH-induced tumor susceptibility have been examined in a great variety of tissues and animals, including man. It was proposed that humans with intermediate and high AHH inducibility carry a higher risk for lung and laryngeal cancer than persons with low inducibility.[103,178] However, these findings could not be confirmed.[143] In various strains of mice, AHH inducibility correlates well with susceptibility to PAH-induced carcinogenesis for some PAHs but not for others.[64,133,135,147] However, none of these studies allows any assessment of whether AHH induction in the target tissue preceded PAH-induced tumor initiation. Even in cases in which positive trends exist between AHH inducibility and susceptibility to PAH-induced tumor initiation, prior treatment with AHH inducers will probably protect against tumor initiation by the PAH. Whether the prior induction of AHH in the target tissue is part of the protective mechanism is unclear.

IN VIVO DISAPPEARANCE OF PAH METABOLITE-DNA ADDUCTS

Mutations and malignant transformations of cells by chemicals may be a consequence of DNA synthesis on parent-strand templates containing unexcised chemically induced lesions. Thus, the ability of a cell to repair the damaged DNA by an error-free pathway could constitute a critical protective mechanism against mutagenesis and carcinogenesis.[33,129] However, Feldman et al.[55,56] and Shinohara and Cerutti[167] reported the persistence of BaP DE-DNA adducts in human alveolar tumor cell A549 and secondary mouse embryo fibroblast, respectively. Dipple and Roberts[47] also noticed the persistence of 7-bromomethylbenz[a]anthracene metabolite-DNA adducts during replication in cell cultures. Cerutti et al.[33,34] observed that significant fractions of BaP metabolite-DNA adducts were still present in the DNA when the alkaline-elution profile of the parent DNA had returned to normal. The persistent lesions could result from the loss of excision repair during prolonged incubation or from the adducts' becoming part of a portion of DNA that cannot be repaired by the excision pathways.[55]

There have been only a few studies on the in vivo disappearance of PAH metabolite-DNA adducts. Before these results are discussed, it should be emphasized that the interpretation of in vivo rates of disappearance of carcinogen-DNA adducts has several limitations. When the SAs of the adducts are based on total DNA content in an organ, the disappearance rates are average cellular values. The disappearance rate in the target cells could be masked by the rates in nontarget cells, especially if the target cells are only a small fraction of the total cells in the organ. In addition, in vivo rates of disappearance of specific activities of adducts cannot be unequivocally equated with enzymatic excision rates, because cell turnover will also result in a decrease in the SA of the adduct.

Eastman and Bresnick[50] examined the disappearance of 3-MC metabolite-DNA adducts in lung and liver of several mouse strains (Table 5-4). In each strain examined, the adduct decreased significantly faster in liver than in lung. The adducts are more persistent in the lungs of A/J and C3H/HeJ mice--strains susceptible to 3-MC-induced pulmonary adenomas--than in the lungs of highly resistant strains. Whether these differences in disappearance rates and initial amounts of 3-MC metabolite-DNA adducts are causally related to differences in tissue and species susceptibility to 3-MC-induced tumorigenesis is unclear.

Anderson and Wilson[9] examined the disappearence of BaP metabolite-DNA adducts in lung and liver of A/HeJ and C57BL/6J mice (Table 5-7). The disappearance rates in A/HeJ were examined at oral doses of 0.011 and 6.0 mg/mouse. The amount of BaP DE-DNA adducts in lung and liver decreased monoexponentially with time at each BaP dose. Although the initial BaP DE-DNA adduct amount at the higher dose was more than 1,000 times larger than that at the lower dose in lung (liver), the half-life of BaP DE adducts in lung (liver) was similar at the two doses (Table 5-7); thus, there is no apparent threshold dose for removal of BaP DE adducts in lung and liver of A/HeJ mice. BaP DE-DNA adducts had a biphasic decay in the lung and liver of C57BL/6J mice (BaP at 6 mg/mouse). Also, the BaP DE adducts in the lung and liver of C57BL/6J mice could be approaching a constant, nondecaying value. Examination of adduct amounts at longer times after the initial dose will be required to determine whether persistent adducts exist. The half-life of the adducts in the terminal phase was similar to that observed in the A/HeJ mice (Table 5-7). In contrast with the results of Eastman and Bresnick[50] with 3-MC, these data do not appear to offer any explanation for the tissue and species differences in susceptibility to BaP-induced neoplasia.

The in vivo disappearance of 11-methylketone metabolite-DNA adducts was examined in lung, liver, and skin of TO mice for 14 d after initial treatment.[2] Rate of DNA turnover was also examined over this same time span. Rates of disappearance of the major adducts in skin and lung could not be measured above the normal rate of DNA turnover, whereas, in the liver, adducts were removed rapidly (half-life, about 2.5 d) relative to the DNA turnover rate. Thus, enzymatic repair of adducts would be occurring in liver, whereas adducts might be persistent in some cell types of skin and lung.[2] Again, whether these apparently persistent adducts of skin and lung are causally related to the initiation of neoplasia in these tissues by 11-methylketone awaits further investigation.

The disappearance of PAH metabolite-DNA adducts has also been examined in skin of mice. Phillips et al.[150] studied the disappearance of DMBA metabolite-DNA adducts in skin of several mouse strains. The half-life of the adducts was 1-2 d in each strain examined. These results do not explain the strain difference in susceptibility to DMBA-induced neoplasia in skin of mice. Similar conclusions were obtained with BaP and 3-MC, although adduct amounts were examined at only two points.[150] Rayman and Dipple[156] examined the formation and disappearance of 7-bromomethylbenz[a]anthracene and 7-bromomethyl-12-methylbenz[a]anthracene metabolite-DNA adducts in skin of Swiss S mice. The adduct amounts of the less carcinogenic 7-bromomethylbenz[a]anthracene were higher and required a longer time to reach maximums--4 d vs. 1 d--after a single topical application of 1 μmol. For both chemicals, the adducts decayed rapidly from the maximum, the half-lives being less than 24 h. The data do not differentiate between the carcinogenic potency of these two PAHs in mouse skin. Pelkonen et al.[146] examined the disappearance of BaP metabolite-DNA adducts in skin and subcutaneous tissue of C3H and C57BL/6 mice. No strain difference was observed in the disappearance in either strain of the BaP 4,5-oxide or the BaP DE adducts. Thus, rates of disappearance of the adducts do not differentiate between the C57BL/6 resistance and the C3H susceptibility to BaP-initiated subcutaneous fibrosarcomas.

In summary, no generalization regarding these data on in vivo disappearance of PAH metabolite-DNA adducts can be made now. In some investigations, extrapolation of the adduct-time curve suggests complete removal of the adducts, whereas adducts appear to persist in other studies. Similar results were obtained in studies of adduct removal in cell cultures. And adduct removal rates differ significantly between tissues susceptible and resistant to PAH-induced neoplasia in some cases and not in others. It is possible that the in vivo results on adduct disappearance rates based on total organ DNA content are misleading, in that PAH metabolite-DNA adducts might be persistent in some cells that constitute only a small fraction of the total cell population.

PAH METABOLITE-DNA ADDUCT AMOUNTS AS A MEASURE OF EFFECTIVE BIOLOGIC DOSE OF SOME PAH TOXICITY

It has been suggested that the amount of carcinogen-DNA binding is a measure of the effective dose of a carcinogen.[113] This proposal is consistent with the somatic-mutation theory of tumor initiation by chemical carcinogens. Although the extent of carcinogen-DNA binding has been successfully used to rank a series of carcinogens, such as PAHs and alkylating agents, for carcinogenic potency,[30,37,88] there have been few, if any, serious attempts to use the amount of carcinogen-DNA adducts formed in the target tissue as a predictive tool for low-dose extrapolation in carcinogenesis. Anderson *et al.*[7] and Gehring and Blau[61] suggested a general scheme for the incorporation of pharmacokinetics in low-dose risk estimation for chemical carcinogenesis. This section discusses the feasibility of using some measure of chemical-induced DNA damage as an effective biologic dose of a carcinogen.

IN VITRO MUTAGENESIS

Several recent studies have examined the extent of PAH-induced DNA damage in cells undergoing mutation and have attempted to relate the PAH-induced mutation frequencies to the amounts of PAH metabolite-DNA adducts. Wigley *et al.*[183] and Newbold *et al.*[138] examined the mutagenicity of benz[a]anthracene, 3-MC, DMBA, BaP, and 7-MBA in a cell-mediated mutagenesis system, using BHK 21 cells to metabolize the PAH and Chinese hamster (V-79) cells as targets for mutation. The frequencies of PAH-induced mutation were not significantly different at equivalent amounts of PAH metabolite-DNA adducts. Yang *et al.*[193] examined the induced-mutation frequency in normal fibroblasts and XP12BE cells as a function of the number of BaP DE-DNA adducts in the cells when they were released from confluence and plated for the expression of 6-thioguanine resistance. The mutation frequency is linearly related to the BaP DE I-DNA adduct amount when the cells were released from confluence.[193] Fahl *et al.*[54] examined the induction of Hist$^+$ reverse-mutation frequencies by BaP DE I, BaP DE II, and 9-hydroxybenzo[a]pyrene in *Salmonella typhimurium* cells (TA 98 and TA 100) as a function of the number of bacterial DNA bases modified by the electrophilic BaP metabolites. The induced-mutation frequencies were linearly related to the DNA adduct amounts in each case (Figure 5-11).

Newbold *et al.*[137] investigated the mutagenicity of BaP DE I and BaP DE II in V-79 cells as a function of BaP DE-DNA adduct amount in the V-79 cells. At sublethal doses of the diol-epoxides, the induced-mutation frequencies were linearly related to the DNA adduct amounts in the cells for both BaP DE I and BaP DE II. The relation of mutation frequency to DNA adduct amount, however, becomes exponential at toxic amounts of BaP DE-DNA adducts. Newbold and Brookes[136] also observed a linear relationship between induced-mutation frequency and the concentrations of BaP DE I in the medium as long as that concentration was sublethal.

Mutation induction by PAHs, as well as by other mutagens and UV radiation, appears to result from the cells' attempt to deal with the unexcised chemical-induced DNA lesions at the time of replication. Moreover, induced-mutation frequencies are linearly related, at least at sublethal doses of the mutagen, to the amounts of mutagen-DNA adducts present when the cells are undergoing replication. This linear relationship makes DNA adduct amounts a good measure of the effective biologic dose of mutagens in in vitro assay systems. Comparisons of the mutagenic potency of PAHs should be based on the slope of the curve relating induced-mutation frequency to PAH metabolite-DNA adduct amounts.

CARCINOGENESIS

In addition to the results obtained in in vitro mutagenic assay systems, the in vivo results discussed below suggest that PAH metabolite-DNA adduct amounts in a known target tissue are a good measure of the effective biologic dose of PAHs for initiation of neoplasia.

Several investigations have shown a positive correlation between the carcinogenicity of a series of PAHs of widely differing carcinogenic potencies and their extent of reaction with DNA.[30,32,68,88,150] This correlation was not observed with the binding of the reactive metabolite of the PAHs to protein and RNA.[30,88] Similarly, the binding of β-propiolactone and similar alkylating agents to DNA, but not to RNA and protein, corresponds to their tumor-initiating potency.[37] Total DNA binding (picomoles of radioactivity associated with DNA per milligram of DNA) was used to rank the carcinogens for carcinogenic potency. Recent studies have suggested that amounts of specific carcinogen-DNA adducts should be used as a measure of effective dose, instead of total DNA binding. For example, for the nitrosamines and nitrosamides, correlation between carcinogenicity and nucleic acid alkylation has been observed only with 06 alkylation of guanine, and not with N7 alkylation of guanine, even though the latter alkylation is approximately 10 times greater than the former.[121,124,144] Current evidence suggests that the bay-region diol-epoxides, such as BP DE, are the ultimate carcinogenic forms for most PAHs. The use of specific carcinogen-DNA adduct amounts, instead of total DNA binding, should improve the ability to rank a series of carcinogens.

Studies with inhibitors of carcinogenesis have shown that tumor response changes quantitatively with PAH metabolite-DNA adduct amount.[6,36,90,184] Swann _et al_.[176] showed that changes in the incidence of dimethylnitrosamine-induced kidney tumors produced by changes in the diet and by treatment with BaP correspond to the changes that these treatments produce in the alkylation of the target-tissue DNA by dimethylnitrosamine.

Janss and Ben[92] found a correlation between the amount of DMBA bound to DNA and the incidence of mammary tumors in rats of different ages.

Several studies have shown that, although the time for appearance of the first tumor was not necessarily dose-dependent, the average time between the administration of a single dose of a carcinogen and the individual appearances of the several tumors was definitely dose-dependent. This increase in the average time for the development of tumors as the dose is lowered led Swann et al.[176] to suggest that the time needed to achieve full malignancy is a function of the amount of the initial preneoplastic lesion, i.e., the formation of carcinogen-DNA adducts.

Most in vivo studies of carcinogen-DNA adduct formation have used total DNA content in an organ to calculate the specific activities of the adducts. However, it is likely that the amounts of adducts formed vary considerably in different cell types. This possibility has the greatest implication for organs (such as the lung) that contain a multitude of cell types and in which the cytochrome P-450-dependent monooxygenase enzymes are localized to a few cell types.[43,165,166] Obviously, the specific activities of carcinogen-DNA adducts in individual cell populations would be a better measure of the effective dose of a carcinogen than the values based on the total DNA content of the organ.

In addition to the extent of carcinogen-induced DNA damage, the capacity of cells to repair such damage and the degree of cell replication are critical in the initiation of carcinogenesis. As with the measurements of SAs of carcinogen-DNA adducts, most in vivo studies of repair of carcinogen-induced DNA damage have been based on total cell populations and thus represent average cellular values. Lewis and Swenberg[118] did study the differential repair of 06-methylguanine in the DNA of rat hepatocytes and nonparenchymal cells (NPCs) after administration of 1,2-dimethylhydrazine. The NPCs are the target cells in 1,2-dimethylhydrazine-induced liver neoplasia. Although the initial alkylation was similar in both cell types, the NPCs repaired 06-methylguanine more slowly than the hepatocytes. This led to a much greater accumulation of the promutagenic lesion in the target cells (NPCs). The rate of cell division was also much higher in the target cells.[119] Thus, in this model system, carcinogen-DNA repair rates and cell division rates were definitely correlated with target-cell susceptibility. Such studies of PAHs are needed to identify the target cells for PAH-induced neoplasia in such organs as the lung.

Carcinogen-DNA adduct amounts, their rates of removal, and cell turnover rates may not be able to explain the difference in organ and species susceptibility to chemical-induced neoplasia. Promotional aspects of carcinogenesis might be required to explain these differences. Determination of whether the initiation characteristics--adduct amounts, DNA-repair rates, and cell turnover rates--can explain the species, strain, and organ differences in susceptibility to PAH-induced neoplasia will require more detailed examination of individual cell types in the organs, such as the studies by Lewis and Swenberg.[118,119]

Even if the initiation characteristics cannot explain organ, species, and strain differences, this does not detract from the use of PAH metabolite-DNA adduct amounts as a measure of the effective biologic dose of a carcinogen in a known target tissue. For low-dose extrapolation of carcinogenic data, for the ranking of a series of similar carcinogens, and for determining the effect on neoplasia of pretreatments that alter the metabolism of a carcinogen, the results discussed above strongly suggest that specific PAH metabolite-DNA adduct amounts are a good measure of effective biologic dose. Adduct amounts in individual cell types of a target organ would probably be an even better measure of effective biologic dose. However, they are not now practical, because the separation of cell types is not generally feasible. We should attempt to incorporate DNA-repair and cell turnover rates into the effective biologic dose of a carcinogen. The use of these initiation characteristics as a measure of effective biologic dose has practical value, because they can usually be studied at doses much lower than those used in bioassay studies.

TRANSCRIPTION AND REPLICATION

Several studies have shown that the functions of DNA during transcription and replication are inhibited by the presence of carcinogen-DNA adducts on the DNA template (Grunberger and Weinstein[77]). Mizusawa and Kakefuda[131] concluded that the BaP DE I-DNA adduct inhibits chain elongation with little effect on initiation of DNA replication. These authors, Yamaura *et al.*,[192] and Hsu *et al.*[87] suggested that elongation of the deoxypolynucleotide chain was terminated at each BaP DE I binding site of the template. Leffler *et al.*[114] demonstrated a progressive inhibition of transcription with increasing amounts of BaP DE I adducts on the template. However, in contrast with the above-mentioned results on replication, Pulkrabek *et al.*[152] concluded that, with some degree of frequency, RNA polymerase can bypass BaP DE I adducts in a template to permit continued chain elongation. They also showed that BaP DE I-modified plasmid DNA could not transfect a receptive *Escherichia coli* strain to antibiotic resistance. The modification of DNA by metabolites of aflatoxin and 2-acetamidofluorene inhibits replication and transcription on the modified template in a similar manner.[77]

Most of the detailed studies of the effects of carcinogen-modified DNA on transcription and replication have been performed in in vitro model systems. However, because the DNA adducts formed in vivo after exposure to BaP are, at least in most cases, the same as those which were used in the in vitro studies, these basic functions of DNA might also be affected after in vivo exposure to BaP. Dose-response studies of formation of PAH metabolite-DNA adducts revealed that adducts will exist after environmental exposure to PAHs. Moreover, recent studies by Anderson et al. (unpublished data) have shown that BaP metabolite-DNA adducts are formed in many organs, both susceptible and nonsusceptible to BaP-induced neoplasia. BaP DE I-DNA adducts were observed in lung, liver, forestomach, brain, kidney, and colon of mice after oral exposure to BaP. Even if the environmental exposures to PAHs are too small to induce neoplasia, the formation of DNA adducts after such exposures could produce aberrations in the transcription (replication) of genetic information in many organs and perhaps lead to subtle toxic effects. Obviously, the amounts of DNA adducts constitute the appropriate measure of effective biologic dose for these considerations.

TABLE 5-1

In Vivo Formation of PAH Metabolite-DNA Adducts

Species[a]	PAH	Dose[b]	Route	Organ	Reference
A/HeJ	BP	23.8	P.O.	Lung, liver	6,184
A/HeJ	BP	11.9	P.O.	Lung, liver, forestomach	3
A/HeJ	BP	0.048-29.7	P.O.	Lung, liver, forestomach	8
C57BL/6J	BP	23.8	P.O.	Lung, liver	9
ICR/Ha	BP	23.8	P.O.	Lung, liver, forestomach	90
A/J	BP	0.05	I.V.	Lung, liver, kidney	51
A/J	3-MC	0.03	I.V.	Lung, liver	51
C57BL/6J	3-MC	0.06	I.V.	Lung, liver	49
A/J, C3H/HeJ, DBA/2J, C57BL/6J	3-MC	0.03	I.V.	Lung, liver	50
C57BL, DBA/2, Swiss	BP, DMBA, 3-MC	0.1, 1.0	Topical	Skin	150
C57BL	DMBA	0.025-1.0	Topical	Skin	150
C57BL/6J, DBA/2, Swiss	BP	0.2	Topical	Skin	11
Ha/ICR	BP	0.004-1.2	Topical	Skin	149
Swiss	BP	0.25	Topical	Skin	14
SENCAR, CD-1	BP	0.1	Topical	Skin	36
C57Bl	BP	1.0	Topical	Skin	41
C57BL/6J	BP	0.4	Topical	Skin	107
C57BL	BP	0.2, 1.0	Topical	Skin	71
C57BL	BP-diols[c]	0.2	Topical	Skin	71
C57BL	3-OH-BP	0.2	Topical	Skin	71
C57BL	3-MC, DMBA	1.0	Topical	Skin	179
TO	11-methylketone	12.9	I.M.	Lung, liver, skin	2
TO	11-methylketone	1.3	I.P.	Lung, liver	2
TO	11-methylketone	1.7	Topical	Skin	1,2,38
Wistar rat	BP	2.9	Topical	Skin	14
Sprague-Dawley rat	BP	1.0, 10.0	I.V.	Lung, liver	23
New Zealand rabbit	BP	198	I.V., I.P.	Lung, liver	d

---

[a] Mouse strain unless otherwise designated.

[b] Micromoles per animal for mice. Micromoles per kilogram for other species. Mouse weight 18-25 g in these studies.

[c] 7,8-Dihydro-7,8-dihydroxy-, 9,10-dihydro-9,10-dihydroxy-, and 4,5-dihydro-4,5-dihydroxy-.

[d] Bixler and Anderson, unpublished data.

TABLE 5-2

Formation In Vivo of BP DE-DNA Adducts

Species[a]	Tissue	Dose,[b] mg/mg	Route	Specific Activity of BP DE-DNA Adducts,[c] pmol/mg of DNA	Reference
A/HeJ	Lung	240	P.O.	6.97	9
A/HeJ	Liver	240	P.O.	6.02	9
A/HeJ	Lung	2.4	P.O.	1.68	9
A/HeJ	Liver	2.4	P.O.	0.98	9
ICR/Ha	Lung	240	P.O.	0.42	90
ICR/Ha	Liver	240	P.O.	0.44	90
ICR/Ha	Forestomach	240	P.O.	0.43	90
C57BL/6J	Lung	240	P.O.	5.80	9
C57BL/6J	Liver	240	P.O.	3.50	9
A/J	Lung	0.5	I.V.	0.14, 0.09	51
A/J	Liver	0.5	I.V.	0.10, 0.08	51
A/J	Kidney	0.5	I.V.	0.03, 0.03	51
New Zealand rabbit	Lung	50	P.O.	0.58	d
New Zealand rabbit	Liver	50	P.O.	0.31	d
New Zealand rabbit	Lung	50	I.P.	0.23	d
New Zealand rabbit	Liver	50	I.P.	0.29	d

---

[a] Mouse strain unless otherwise designated.

[b] Mice assumed to weigh 25 g. In all P.O. studies in mice, two equal doses of [^{3}H]BP were given 2 h apart. Values represent total dose.

[c] Values represent sums of BP DE I and BP DE II adducts. Adducts determined 48 h after [^{3}H]BP dose, except for studies in A/J mice, in which first value is 4 h after I.V. dose and second is 24 h after dose. Average of at least two determinations.

[d] Bixler and Anderson, unpublished data.

TABLE 5-3

In Vivo Formation of BP Metabolite-DNA Adducts in Lung and Liver of Rats

Tissue	Dose,[a] μmol/kg	Specific Activity,[b] fmol/mg of DNA: Total DNA Binding	Specific Activity,[b] fmol/mg of DNA: BP-phenol-oxide-DNA Adduct
Lung	1.0	133 ± 11	55.5 ± 6.0
Liver	1.0	730 ± 220	48.2 ± 5.5
Lung	10.0	680 + 165	178 ± 41
Liver	10.0	2,500 ± 610	168 ± 25

---

[a] I.V. injection.

[b] Determined 1 h after I.V. injection of [^{3}H]BP. Data from Boroujerdi *et al.*[23] Mean ± S.E. for three experiments.

TABLE 5-4

Binding of 3-MC to DNA in Lung and Liver of Mice

Strain	Tissue	In Vivo Exposure Time[a]	Specific Activity,[b] fmol/mg of DNA: Total[c]	Specific Activity,[b] fmol/mg of DNA: Nucleoside-Bound Adducts[d]
A/J	Lung	4 h	113	44.9
		7 d	74	30.0
		28 d	106	15.9
	Liver	4 h	135	5.5
		7 d	96	5.5
		28 d	96	0
C3H/HeJ	Lung	4 h	76	17.6
		7 d	43	8.3
		28 d	43	6.1
	Liver	4 h	45	4.4
		7 d	20	0.6
		28 d	29	0
DBA/2J	Lung	4 h	68	16.3
		7 d	35	6.3
		28 d	24	1.1
	Liver	4 h	64	4.8
		7 d	50	1.3
		28 d	28	0
C57BL/6J	Lung	4 h	173	16.1
		7 d	103	2.7
		28 d	83	1.7
	Liver	4 h	139	9.7
		7 d	135	0
		28 d	107	0

---

[a] Time of sacrifice after I.V. injection of [^{3}H]3-MC (12.6 mg/mouse).

[b] Data from Eastman and Bresnick.[50]

[c] Mean of 6-10 mice (3-5 determinations). Fairly large individual variation was observed with S.D. of ± 25% of mean.

[d] Peaks V and VI in Figure 5-4. Values obtained by multiplying % of radioactivity eluting from Sephadex LH20 as peaks V and VI by total DNA-associated radioactivity.

TABLE 5-5

Effect of AHH Inducters on In Vivo Formation of BP DE-DNA Adducts and on Total DNA-Associated Radioactivity in Mice[a]

Strain	Treatment[b]	Tissue	Specific Activity[c] (pmol/mg of DNA) of BP DE-DNA Adducts in Treated Mice, % of control	Total DNA-Associated Radioactivity, % of control
A/HeJ	β-NF (Group A)	Lung	15	43
A/HeJ	β-NF (Group A)	Liver	0	67
A/HeJ	β-NF (Group B)	Lung	8	46
A/HeJ	β-NF (Broup B)	Liver	0	48
A/HeJ	TCDD	Lung	5	73
A/HeJ	TCDD	Liver	0	182
A/HeJ	Aroclor 1254	Lung	9	123
A/HeJ	Aroclor 1254	Liver	0	101
ICR/Ha	β-NF	Lung	16	42
ICR/Ha	β-NF	Liver	7	55
ICR/Ha	β-NF	Forestomach	16	40

---

[a] Untreated or treated mice were killed 48 h after oral dose of [^{3}H]BP (6 mg/mouse). DNA isolated from tissue was enzymatically digested, and deoxyribonucleosides were chromatographed on HPLC. Specific activity of BP DE-DNA adducts calculated from HPLC chromatogram.

[b] Mice were fed βNF (3 mg/g of diet) for 2 wk before [^{3}H]BP administration. See text for discussion of Group A and B animals. Animals treated with TCDD (80 nmol/kg) 4 d before [^{3}H]BP administration. Aroclor 1254-induced mice received inducer (500 mg/kg) 48 h before administration of [^{3}H]BP.

[c] Data for A/HeJ mice from Wilson et al.[184] Data for ICR/Ha mice from Ioannou et al.[90] Zero means adduct not detected in treated animals.

TABLE 5-6

Effects of Pretreatment with TCDD on In Vivo Covalent Binding of BP to Mouse Epidermal DNA, RNA, and Protein

Strain	BP Dose,[a] mg/mouse	Time,[b] h	Hydrocarbon Bound to Macromolecules (pmol/mg) in Treated Animals,[c] % of control		
			DNA	RNA	Protein
SENCAR	25	3	250	217	255
SENCAR	25	24	278	133	260
CD-1	50	24	318	100	242

---

[a] Mice received single topical application of [^{3}H]BP. Animals received single topical application of TCDD (1 mg in 0.2 ml of acetone) or acetone 72 h before carcinogen.

[b] Time of sacrifice after application of BP.

[c] Data from Cohen *et al.*[36]

TABLE 5-7

In Vivo Disappearance Rates of BP DE-DNA Adducts in Lung and Liver of Mice

Strain	Tissue	P.O. Dose, mg/mouse	Half-life for Disappearance of BP DE-DNA Adducts,[a] days
A/HeJ	Lung	6.0	17
A/HeJ	Lung	0.012	19
A/HeJ	Liver	6.0	9
A/HeJ	Liver	0.012	16
C57BL/6J	Lung	6.0	19
C57BL/6J	Liver	6.0	14

---

[a] Animals sacrificed at intervals of 10 h to 28 d after oral dose of [^{3}H]BP. Specific activity of BP DE-DNA adducts decreased monoexponentially in lung and liver of A/HeJ mice. There was biphasic decay of adducts in lung and liver of C57BL/6J mice. Half-life of terminal phase given here. Values are sums of BP DEI- and BP DEII-DNA adducts. Data from Anderson and Wilson.[9]

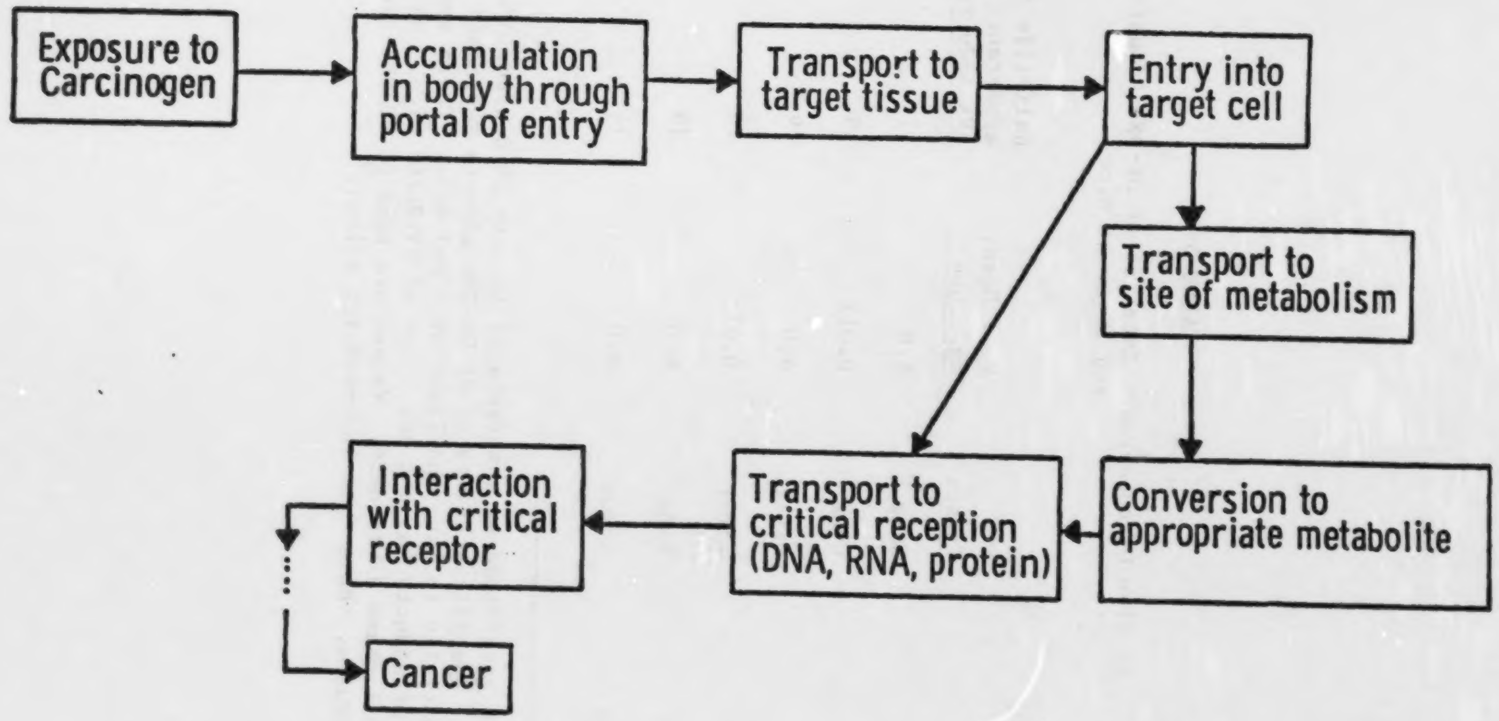

Exposure to Carcinogen
Accumulation in body through portal of entry
Transport to target tissue
Entry into target cell
Transport to site of metabolism
Conversion to appropriate metabolite
Transport to critical reception (DNA, RNA, protein)
Interaction with critical receptor
Cancer

FIGURE 5-1. Schematic of pharmacokinetic factors involved in tumor initiation.

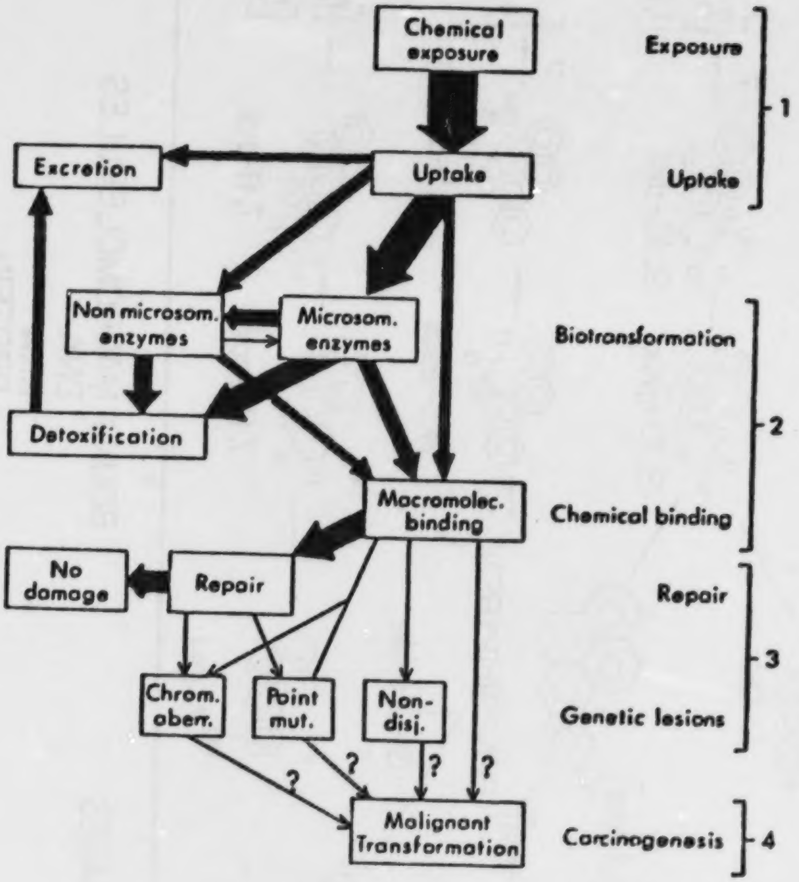

Chemical exposure
Exposure
1
Excretion
Uptake
Uptake
Non microsom. enzymes
Microsom. enzymes
Biotransformation
Detoxification
2
Macromolec. binding
Chemical binding
No damage
Repair
Repair
3
Chrom. aberr.
Point mut.
Non-disj.
Genetic lesions
?
?
?
?
Malignant Transformation
Carcinogenesis
4

FIGURE 5-2. Sequence of possible events from exposure to carcinogenesis.

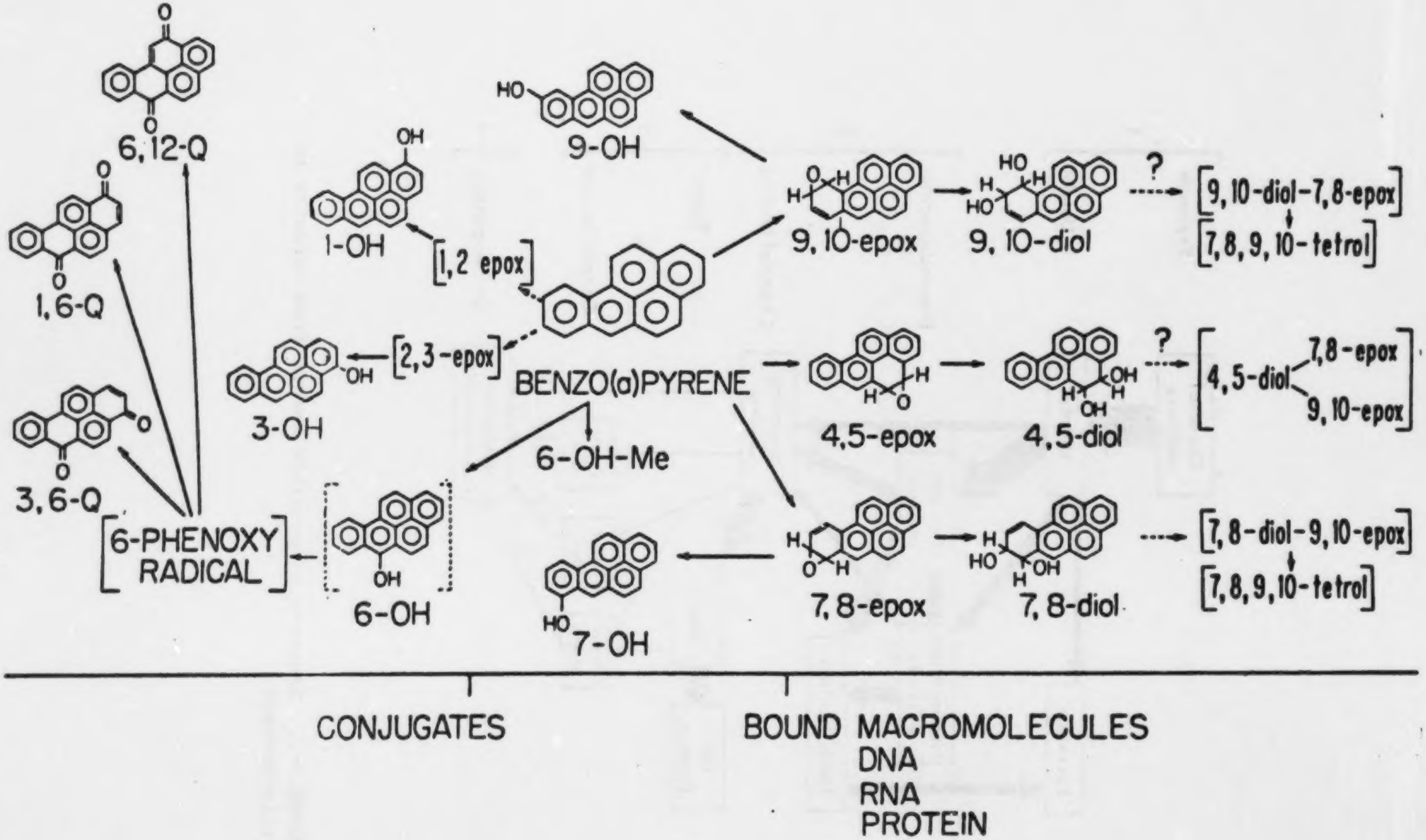

6,12-Q
1,6-Q
3,6-Q
6-PHENOXY RADICAL
1-OH
[1,2 epox]
9-OH
3-OH
[2,3-epox]
BENZO(a)PYRENE
6-OH-Me
6-OH
7-OH
9,10-epox
9,10-diol
?
[9,10-diol-7,8-epox]
[7,8,9,10-tetrol]
4,5-epox
4,5-diol
?
4,5-diol
7,8-epox
9,10-epox
7,8-epox
7,8-diol
[7,8-diol-9,10-epox]
[7,8,9,10-tetrol]
CONJUGATES
BOUND MACROMOLECULES
DNA
RNA
PROTEIN
HO
OH
H
O

FIGURE 5-3. Composite of metabolic products of benzo[a]pyrene.

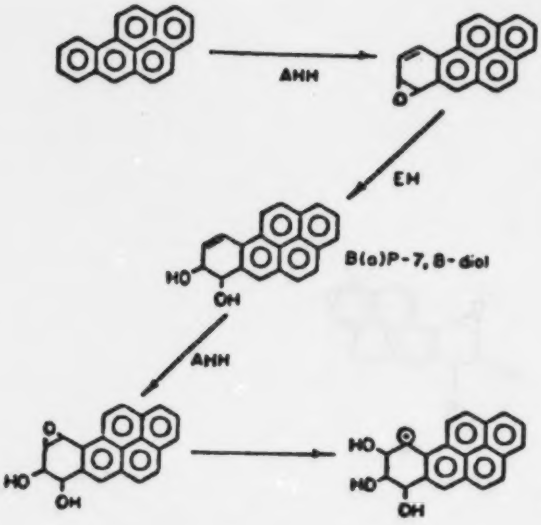
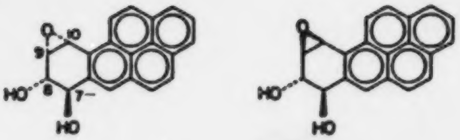

AHH
EH
B(a)P-7,8-diol
HO
OH
AHH
HO
OH
HO
HO
OH

FIGURE 5-4. Sequences in formation of highly electrophilic 7,8-diol-9,10-epoxide.


O
10
9
HO
8
7
HO
O
HO
HO

I II

FIGURE 5-5. Structures of BPDE; both isomers represent enantiomeric mixtures.

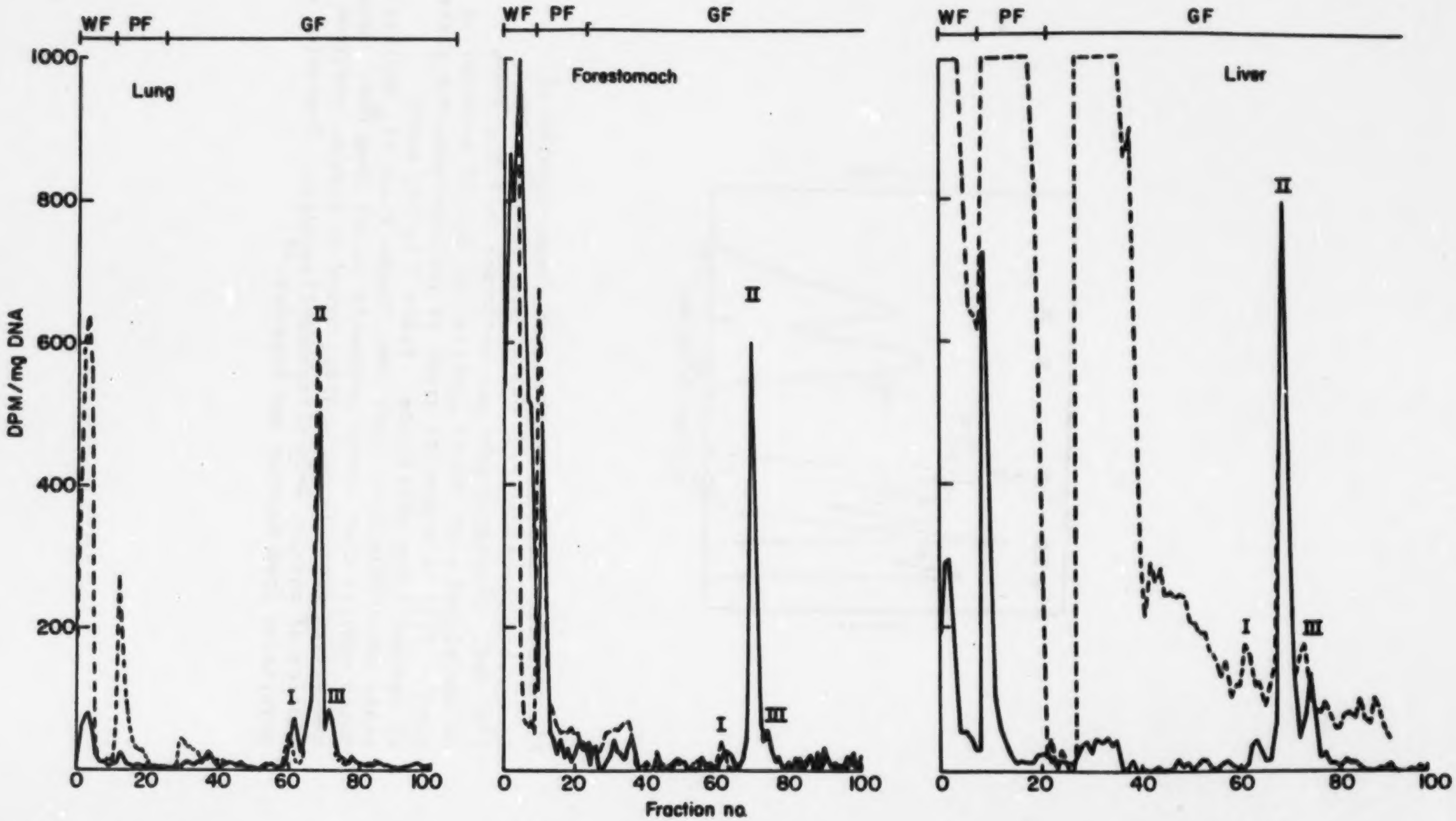


FIGURE 5-6. Comparison of HPLC analysis of nucleoside-bound BP metabolites obtained from DNA isolated by precipitation (-----) and hydroxyapatite (——) procedures. When curves are identical, only solid curve is illustrated. Mice were killed 48 h after p.o. dose of ^{3}H[BP] (3 mg/mouse). DNA isolated from lung, forestomach, and liver was enzymatically digested, and deoxyribonucleosides were chromatographed on C_{18}-silica column with linear aqueous-methanol gradient. WF, water fraction; PF, pregradient fraction; GF, gradient fraction. Peaks I, II, and III observed in gradient fraction are discussed in text. Specific activities of various peaks are given in Table 5-2. Reprinted with permission from Adriaenssens et al.;[3] copyright Academic Press.

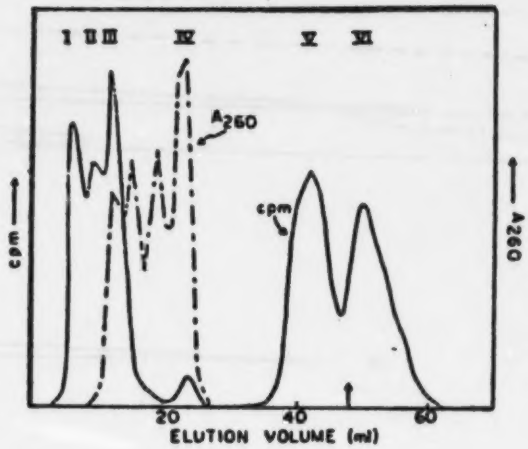


FIGURE 5-7. Sephadex LH-20 column chromatography of deoxyribonucleosides from DNA of mice treated with [^{3}H]-3MC. Chromatography was performed on 0.9 x 30-cm column eluted with 140-ml gradient of 30-100% methanol in water. This is composite graph to indicate possible peaks of radioactivity obtainable. Peaks I to IV, early peaks obtainable from liver DNA. Peaks V and VI, nucleoside-bound adducts that appear maximally in A/J lung DNA. A_{260}, unmodified deoxyribonucleoside; arrow at bottom, position of elution of marker, 4-(p-nitrobenzyl)pyridine. Reprinted with permission from Eastman and Bresnick.[49]

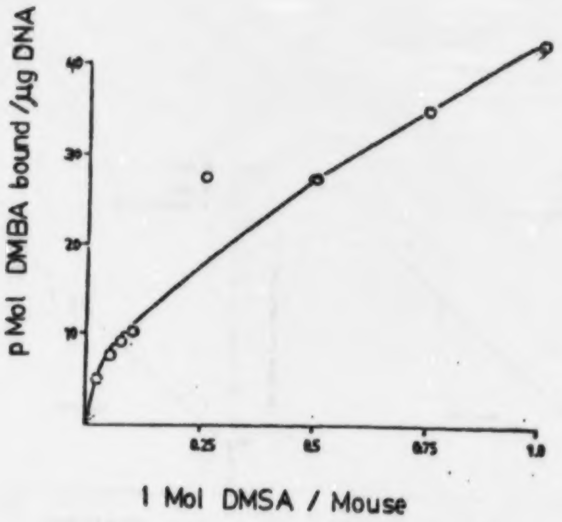


FIGURE 5-8. Binding of 7,12-dimethylbenz[a]anthracene to DNA of skin of C57BL mice 19 h after topical application. Binding determined from elution profiles of DNA hydrolysates prepared from treated skin. Reprinted with permission from Phillips *et al.*[150]

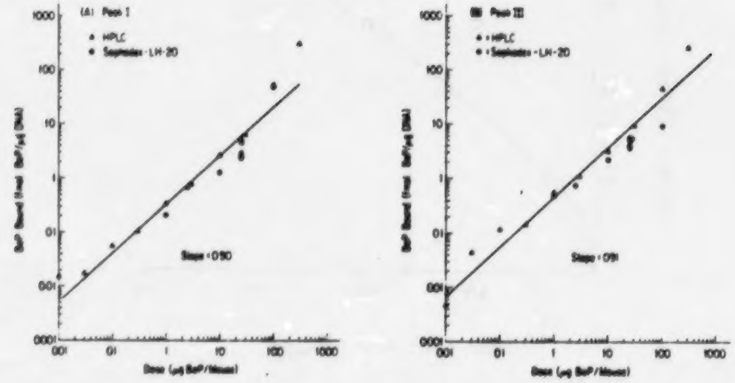


FIGURE 5-9. Dose dependence of BaP adduct formation in epidermal DNA. Groups of mice, 15-20 each, were treated topically with 1, 2, 5, 10, 25, and 100 μg of [^{3}H]BP and sacrificed 24 h later. Enzymatically digested epidermal DNA was chromatographed on Sephadex LH-30 or Waters C_{18}-μBondapak column. A, dose dependene of peak I formation; B, dose dependence of peak III formation. Reprinted with permission from Pereira _et_ _al_.[149]

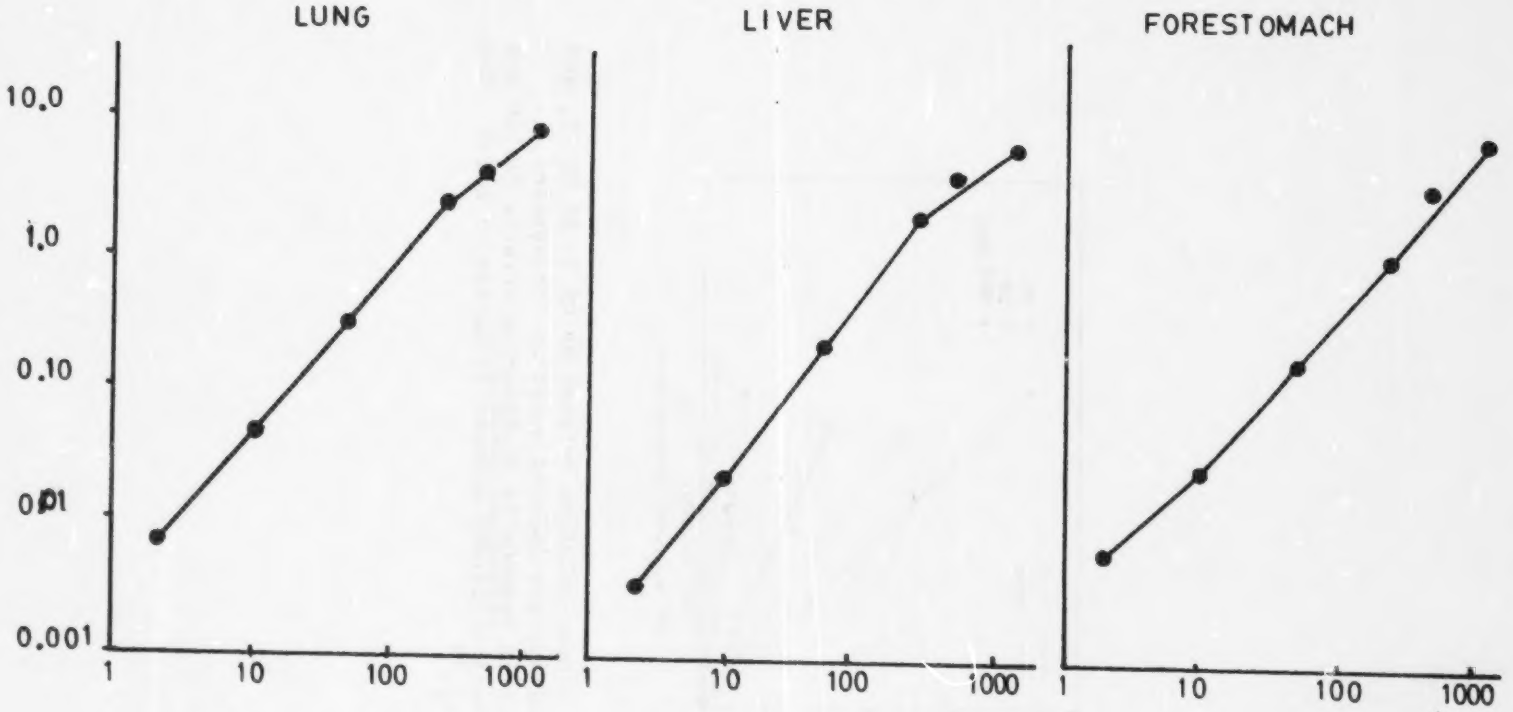


FIGURE 5-10. Dose-response relation for BP DE-DNA adducts in lung, liver, and forestomach of A/HeJ mice. BP was administered orally to mice at 2-1,351 μmol/kg. Mice were sacrificed 48 h later. DNA isolated from tissue was enzymatically digested, and deoxyribonucleosides were chromatographed on HPLC. Specific activities of BP DE adducts were determined as described in Adriaenssens _et al_.[3]

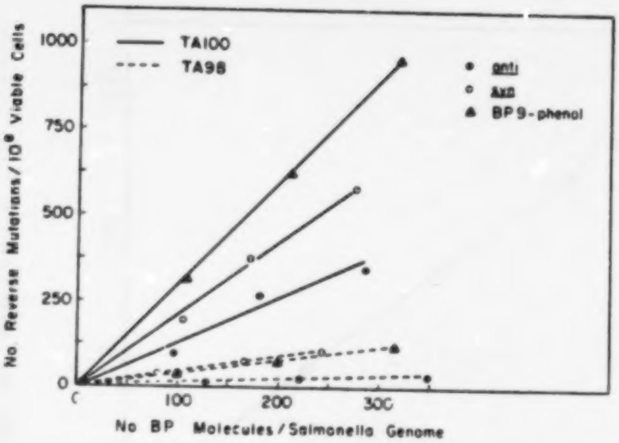


FIGURE 5-11. Linear relation between BP DE I, BP DE II, and 9-hydroxybenzo[a]pyrene-induced mutation frequencies at histidine locus in *Salmonella typhimurium* strains TA 100 and TA 98 and BP metabolite-DNA adducts in bacterial cells. Data from Fahl *et al.*[54]

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6

POLYCYCLIC AROMATIC HYDROCARBONS IN FOOD AND WATER AND THEIR METABOLISM BY HUMAN TISSUES

This chapter deals with the relation of PAHs to human metabolism. Specifically, its purposes are to collate a large volume of literature dealing with the capacities of a number of human tissues to interact with and biotransform selected PAHs; to define, where possible, the effects of these compounds on human tissues; and to examine the principal sources of human exposure to PAHs through food and water.

PAH METABOLISM BY HUMAN TISSUES

The abilities of various human tissues to metabolize PAHs have been extensively studied, with emphasis on the chemical biotransformations that are catalyzed by tissues that can be readily sampled (such as blood cells, skin, and placenta) or that can be biopsied or cultured (such as fibroblasts, liver, and intestinal and tracheobronchial epithelium). The chemical biotransformations of selected PAHs that such tissues carry out are in general qualitatively similar to those demonstrated in animal tissues, although there are considerable species and organ differences in catalytic activities of relevant enzymes. These differences may be great enough to preclude comparative generalizations; and for the most part the relation between in vitro and in vivo enzymatic activities is unclear. Moreover, it is apparent from the findings reviewed in this chapter that the enzymatic capacity to biotransform PAHs to ultimate carcinogens in various tissues is not necessarily correlated with the demonstrated ability of PAHs to produce cancers in those tissues.

SKIN

That benzo[a]pyrene hydroxylase can be induced in cultured human skin was first demonstrated in 1972.[103] Foreskins from children who were circumcised 2-4 d after birth were shown to contain an enzyme that hydroxylates the carcinogen benzo[a]pyrene (BaP), and induction of the enzyme (by a factor of 2-5) was demonstrated when the foreskins were cultured for 16 h in the presence of 10 μM benz[a]anthracene. Among a group of 13 skin samples studied, control enzymatic activities extended over a threefold range and were not correlated with race, age of mother, or medications given to mother or infant.[2] The enzyme had an absolute requirement for NADPH and molecular oxygen and was completely inhibited by CO; these findings suggested the involvement of a species of cytochrome P-450 in the hydroxylation reaction. The presence of this heme protein in low concentrations in cutaneous tissue was later demonstrated by Bickers et al.[22] Coal-tar products, which are widely used in dermatologic practice in conjunction with exposure to ultraviolet light (e.g., the Goeckerman regimen for psoriasis[62,63]), were also shown to induce aryl hydrocarbon hydroxylase (AHH) significantly in patients with dermatologic disease where the coal tar was applied, but not in skin distant from the site of application.[21] In concurrent studies in neonatal rats,[21] although (as in humans) distant skin sites were unaffected by the coal-tar application, AHH activity in the livers of treated animals increased to more than 20 times the control values. Among five identifiable constituents of coal tar studied[21] for their AHH-inducing properties in human skin, BaP was the most potent; pyrene and anthracene also caused significant induction of the enzyme. In isolated cultured human hair follicles, Vermorken et al.,[185] using radioactive BaP as substrate, not only demonstrated the presence of the hydroxylase, but identified the formation of the 3-OH, 7,8-dihydro-7,8-dihydroxy, and 9,10-dihydro-9,10-dihydroxy metabolites of this PAH.

BaP clearly has cytotoxic effects on cultured human skin fibroblasts, although relatively high concentrations are required for cytotoxicity. Milo et al.[123] studied the influence of the three carcinogenic PAHs, 7,12-dimethylbenzanthracene (7,12-DMBA), 3-methylcholanthrene (3-MC), and BaP on mixed-function oxidase* activity, cell proliferation kinetics, and DNA damage in cultured fibroblasts. They found that only BaP, at 10 μg/ml or higher, affected all the cellular metabolic characteristics examined. 7,12-DMBA at 6 μg/ml or higher induced the mixed-function oxidase system and stimulated DNA synthesis; 3-MC at concentrations as high as 15 μg/ml produced no significant cellular alterations. Similarly, 5-fluoro-7,12-DMBA, anthracene, and phenanthrene had no effects on these human cells. The authors concluded that BaP alone could initiate all the biochemical events probably necessary to trigger transformation of human cells in vitro.

PAH-induced cytotoxicity to cultured human fibroblasts has also been demonstrated by Strniste and Brake[172] and Aust et al.[8] In the former study, normal fibroblasts and xeroderma pigmentosum (XP) cells were used, and BaP was "activated" by light radiation (near ultraviolet), rather than enzymes. Photoactivation (at 300-400 nm) of BaP produced at least three identifiable quinones (1,6-, 3,6-, and 6,12- isomers), as well as more hydrophilic products, depending on the duration of light exposure. Formation of these products was oxygen-dependent. The irradiation products led to several types of DNA damage, with covalently bound hydrocarbons constituting the major lesion under all conditions studied. XP cells were more sensitive to damage than normal cells (by a factor of 1.7-2), and sensitivity increased by a factor of 10 when long-wavelength ultraviolet light was used. 7,12-DMBA, 3-MC, and BaP were also examined; the order of phototoxicity was 7,12-DMBA > BaP > benzo[e]pyrene > 3-MC.

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*"Mixed-function oxidase" refers to the NADPH-dependent enzyme complex containing cytochrome P-450s in the membranes of the endoplasmic reticulum, which catalyzes the oxidation of numerous structurally diverse molecules, including drugs, steroid hormones, and carcinogens.

In the study of Aust et al.,[8] a human epithelial cell-mediated cytotoxicity and mutagenicity assay system for BaP was developed with human fibroblasts as the target cells. Lethally x-irradiated human kidney-carcinoma epithelial cells were cocultivated with human XP skin fibroblasts (XP12BE) lacking excision-repair capability for BaP-DNA adducts. Under defined conditions, the frequency of mutation to 6-thioguanine resistance and PAH binding to DNA were shown to be concentration-dependent. Two principal BaP-DNA adduct peaks could be identified--a major peak consistent with an adduct standard synthesized from the anti-isomeric 7,8-dihydrodiol-9,10-epoxide of the hydrocarbon and a minor peak consistent with the syn-isomeric form of this metabolite. The results are consistent with those in other reports on BaP adducts formed in human explant tissue from lung,[161] colon,[10] esophagus,[68] and bronchus,[75] and they represent an advance in the development of sensitive assay systems for detecting biologic responses to human epithelial-cell activation of BaP.

Direct neoplastic transformation of human fibroblasts by carcinogens has also been demonstrated. In the study of Kakunaga,[79] normal human adult fibroblasts exposed to the carcinogen 4-nitroquinoline 1-oxide underwent malignant transformation in a process requiring numerous cell divisions. When injected into athymic (nude) mice, the transformed cells produced solid tumors at the site of inoculation. Because it could not be metabolically activated by the target cells used, 3-MC was unable to effect transformation; the use of other PAHs and induced microsomes with high concentrations of cytochrome P-450 to activate 3-MC was not examined. Normal human foreskin cell populations were neoplastically transformed in studies by Milo and DiPaolo[124,125] with a number of non-PAH carcinogens; and treatment with a tumor promoter alone (phorbol ester) has been shown[90] to induce neoplastic transformation in fibroblasts from humans genetically predisposed to cancer (familial polyposis of the colon). Thus, it can be inferred that cells already in an "initiated state" as a result of a genetic defect represent a novel fibroblast system that may provide a means for exploring separately the roles of initiators and promoters in carcinogenesis. Painter[135] used HeLa cells to develop a rapid screening test to detect agents that damage human DNA. The test measures thymidine uptake into the cells at various times (principally 1-2 h) after treatment with a presumptive carcinogen or mutagen. In this test system, BaP was inert unless metabolically activated by incubation with rat-liver microsomes. Brookes and Duncan[26] compared the effects of PAHs on primary human embryo cells and HeLa cells. Fibroblasts from skin, lung, muscle, and gut were cultured and treated with ^{3}H-labeled BaP and 7,12-DMBA.

Both hydrocarbons were metabolized in the cultures, 7,12-DMBA more slowly than BaP; among the cell types studied, lung fibroblasts metabolized the compounds more efficiently than others and retained this capacity well in subculture. The binding of BaP and 7,12-DMBA to DNA, RNA, and protein of these primary lung-derived fibroblasts was also studied (metabolism of each hydrocarbon exceeded 75% during the 48-96 h of treatment) and was found to be significantly greater for BaP than for 7,12-DMBA. The data in this study also established parallelisms, at least for BaP, between hydrocarbon binding to cellular macromolecules in fibroblast cultures derived from mouse embryos and those derived from human lung cells. Such parallelism is of more than casual interest, in view of the susceptibility of the mouse to hydrocarbon carcinogenesis and the known correlation between hydrocarbon-DNA binding and cancer-producing activity in mouse skin.

The effects of pyrene and BaP in the human diploid fibroblast culture WI-38 were studied by Weinstein et al.[188] Neither caused significant damage (compared with controls), as assessed by mitotic index or chromosomal breaks after 1-h pulse exposures. However, metabolic activation of BaP with microsomes resulted in a dramatic decrease in mitotic index and a significant increase in breakage. Microsomal incubation did not alter the inertness of pyrene in this test system. Freeman et al.[60] have made interesting observations on comparative aspects of hydrocarbon metabolism in skin epithelial and fibroblast cultures. A comparison of the ability of epithelial-cell colonies and of fibroblast colonies from the same 13 subjects to metabolize BaP to a water-soluble form demonstrated clearly the markedly greater metabolizing capacity of epithelial cells. There was a 20-fold difference in this capacity of epithelial cells; within individual subjects, the ability of epithelial cells to metabolize the PAH exceeded that of fibroblasts by as much as a factor of 40. There appeared to be a major effect of culture age (6-55 d) on the ability of epithelial cells to metabolize BaP.

A direct toxicity of BaP to normal human epithelial-cell cultures has been described by Dietz and Flaxman.[50] This toxicity was reflected in a dramatic reduction in epidermal-cell outgrowth, a decrease in mitotic indexes, a loss of the well-ordered cell relationships, and the early appearance of giant cells ranging in diameter from 100 to 200 μm. In a clinical study that clearly could not be carried out today, Cottini and Mazzone[45] (in 1939) applied a 1% solution of BaP daily (up to 120 d) to the skin of 26 normal subjects and patients with various dermatologic disorders and examined the gross and histologic consequences. The sequential epidermal changes, of which gross pigmentation and verrucae were the most frequent, and histologic alterations (which regressed within several months when treatment was terminated) led the authors to conclude that "benzopyrene, if applied to human skin for protracted periods, would be carcinogenic as it is in animals."

The metabolism of benz[a]anthracene, 7,12-DMBA, and BaP by human mammary epithelial-cell aggregates in culture has been investigated by Grover *et al.*[66] with nonneoplastic tissue obtained from eight patients undergoing reduction mammoplasty. All three PAHs were metabolized to water-soluble and organic-solvent-soluble products; the latter included K-region and non-K-region dihydrodiols. The major dihydrodiols detected as metabolites of the parent PAHs were the 8,9-dihydrodiols of BaP and 7,12-DMBA and the 9,10-dihydrodiol of BaP. The hydrocarbons bound to the proteins and DNA of the epithelial cells, but there were wide differences between different PAHs in extent of binding between tissue preparations from different patients. Some of the PAH-deoxyribonucleoside adducts formed from 7,12-DMBA and BaP appeared to have been produced through reactions of bay-region diol-epoxides with DNA, but little reaction with DNA was detected in tissue preparations treated with BaP.

Unscheduled DNA synthesis induced by DNA-damaging chemicals has been measured in nonreplicating human fibroblasts by autoradiographic methods that are not readily applicable to organotypic epithelial-cell cultures. To evaluate the range of chemical sensitivity and DNA-repair responses of human skin epithelial cells, Lake *et al.*[95] developed a semiquantitative in vitro method for measuring unscheduled DNA synthesis in normal foreskin epithelial cells. On serial subculture of organotypic primary skin cultures, the unscheduled DNA synthesis response elicited by 3-MC decreased in parallel with the ability of cells to metabolize PAHs to water-soluble metabolites. The working hypothesis was that procarcinogens that are efficiently activated by human skin-specific metabolism will be detected with unscheduled DNA synthesis as an end point.

LIVER AND INTESTINE

Obana *et al.*[130] analyzed quantitatively and qualitatively the PAH content of samples of human liver and fatty tissue. Six samples of liver and 10 samples of fat were obtained at autopsy from 10 persons who died of unstated causes (although the tissues were reported to be "free from cancer"). Smoking habits, occupations, etc., were not described. The tissue samples analyzed were quite large (40-120 g), and the PAHs were determined without complex pretreatment. Table 6-1 shows the analytic results for liver, and Table 6-2 the comparable data for fat tissue. Note that PAH concentrations are expressed as parts per trillion (ppt), not parts per billion (ppb), and are in general extremely low. Nevertheless, the data indicate that, on the average, the PAH concentration in liver was one-third that in fat. Pyrene had the highest concentration, followed by anthracene. Although the number of samples was small, no sex or age differences were evident. The known carcinogens benz[a]anthracene and dibenz[ah]anthracene were not detected in either tissue. However, BaP was detected in small amounts (20 ppt) in both liver and fat. This finding should be compared with that of Tomingas *et al.*,[178] who detected BaP at 1-15,000 ppb in human bronchial-carcinoma tissue (24 samples). Obana et al.[130] called attention to the fact that the PAHs in the human tissues they examined were different, in both concentration and composition, from the PAHs that had been identified in marine samples. For example, pyrene was found in oysters at 7-52 ppb and in Wakame seaweed at 12-41 ppb; the comparable figures for BaP were 0.3-2.6 ppb and 0.6-9 ppb, respectively. Moreover, although pyrene was the most abundant PAH in all cases, the next most abundant in the human tissues was anthracene, whereas in the marine samples the next most abundant were benzo[e]pyrene and benzo[b]fluoranthene. These qualitative and quantitative distinctions, especially the marked concentration differences between nontumorous[130] and tumorous tissues[178] and between a common food source in the area and the human tissue samples, need to be recalled in evaluating the importance of the food content of PAHs, as well as the role of malignant pathology, when trying to determine the significance of the body or tissue burden of these hydrocarbons.

The liver contains the highest concentration of cytochrome P-450 in the body. The activity of the pathway by which heme, the prosthetic group of this heme protein, is synthesized can be greatly induced by a host of foreign chemicals and can approach the rates of heme synthesis in erythroid cells; and the enzymatic capacity of hepatic cells to carry out the biotransformations that characterize the great variety of PAH metabolites formed in vitro, and probably in vivo, has been well defined. Only selected PAH transformations catalyzed by liver cells are reviewed here, with some emphasis on the relationships of PAH- and drug-metabolizing capabilities and on recent data indicating that carcinogen metabolism may be increased by direct actions on relevant membrane-bound enzymes, as well as by the conventionally assumed process of increased de novo synthesis of enzyme protein, i.e., induction.

Dybing et al.[54] have examined the in vitro metabolism and metabolic activation of several carcinogenic PAHs in subcellular fractions from seven human livers. The patients all suffered from total cerebral infarction and were serving as potential kidney donors (maintained temporarily by life-support systems) at the Huddinge University Hospital in Sweden. At the appropriate time, liver extirpation was performed; within 20 min after the procedure, perfusion had been completed and the tissue frozen in liquid nitrogen. This study may mimic the enzymatic properties of human liver cells in the living subject as closely as experimentally possible, other than by direct biopsy or surgery in a living patient. Because of the unique source of the tissue studied, some of the data merit recording here. Microsomal cytochrome P-450 content (seven livers) was 0.16-0.60 nmol/mg of protein, with a mean ± S.D. of 0.36 ± 0.15, and AHH activity averaged 175 ± 138 pmol/mg of protein per minute; one sample had a value of 483 pmol/mg. These activities are approximately the same as those in liver microsomes of untreated mice and rats. AHH activities expressed per nanomole of cytochrome P-450 varied by a factor of 2.8 among the seven liver samples.

Conney and co-workers[27,28,40,41,73,81,82,94,156,157,174] conducted a series of studies of direct liver-cell metabolism of carcinogens and compared such metabolism with that of drugs. They established that carcinogen metabolism may be increased not only through enzyme induction, but through enzyme activation as well. They compared the oxidative metabolism of BaP with that of antipyrine, hexobarbital, coumarin, zoxazolamine, and 7-ethoxycoumarin in 32 adult liver samples obtained at autopsy some 8-20 h after death. When enzyme activity for one substrate was plotted against enzyme activity for a second substrate for each of the 32 liver samples, significant correlations were found. For example, for BaP paired against antipyrine, hexobarbital, zoxazolamine, and coumarin, the correlation coefficients were 0.85, 0.72, 0.69, and 0.57, respectively. Some drug-drug metabolizing activities also showed a high correlation (e.g., antipyrine and hexabarbital, r = 0.79; antipyrine and coumarin, r = 0.72), whereas in other instances, metabolizing capacities did not have a high correlation, e.g., carcinogen vs. drug (BaP and 7-ethoxycoumarin, r = 0.35) and drug vs. drug (e.g., hexobarbital and 7-ethoxycoumarin, r = 0.37). The findings raise the possibility that an in vivo drug-metabolism assay (e.g., a plasma disappearance-rate study of a suitable test drug) might predict some carcinogen-metabolizing capabilities of a person and suggest the presence in humans of multiple monooxygenase systems for the substrates studied, as well as their heterogeneous distribution in the population. Individual differences in the rates of metabolism of BaP (7-fold) in these and other liver samples studied[41] were considerable, although they did not approach the known species differences[40] in rates of metabolism of drugs.

The effects of PAH administration on the metabolic disposition of a specific carcinogen, such as BaP, have not been studied in humans, but Schlede *et al.*[156,157] recently examined the metabolic disposition of radiolabeled BaP in rats, and the results probably can be extrapolated to humans. Pretreatment of rats with unlabeled BaP greatly increased the plasma disappearance rate of a tritiated dose of the same compound given intravenously; the effect was especially marked during the first 5 min after the intravenous dose of the radiolabeled material, and the increased rate lasted for at least 6 h. This effect of pretreatment with the compound was paralleled by a lower concentration of [^{3}H]BaP in brain, liver, and fatty tissues; similar but more varied results were observed in lung tissue. These influences of BaP pretreatment on a later intravenous dose of the ^{3}H-labeled chemical were also observed when the radiolabeled PAH was administered orally. 3-MC and 7,12-DMBA pretreatment of animals produced comparable effects on the metabolic disposition and tissue contents of radiolabeled BaP. Pyrene, anthracene, and phenobarbital had little or no such effect on the in vivo disposition of this compound. In other studies, the biliary excretion of [^{14}C]BaP was shown to be increased by pretreatment with the unlabeled compound; however, no increase in excretion into bile of the ^{14}C-labeled metabolites of BaP was observed after prior administration of this PAH. These findings suggest that conversion of

BaP to its metabolites may be the rate-limiting step in the biliary excretion of the compound. Phenobarbital had no effects on the pharmacokinetics (plasma disappearance) of [^{14}C]BaP and its metabolites; this drug might stimulate the conjugation of hydroxylated BaP derivatives before their excretion into bile. The relevance of these findings to humans is related not only to the (probably) qualitatively similar pharmacokinetics of such a chemical as BaP--particularly its extensive excretion via bile--but also to the potentially important use of selected drugs, singly or multiply, to increase disposal of PAHs and their metabolites from the body by stimulating conjugation and biliary excretion and by increasing otherwise innocuous metabolic biotransformations.

Prough *et al*.[143] have also studied BaP metabolism in human liver, kidney, and lung, and they characterized the metabolites formed by HPLC techniques. Tissue samples were obtained within 1-5 h after death, and assays were completed within the succeeding 2-4 h. In the analysis of metabolites formed from BaP, quinones, three classes of dihydrodiols, and two classes of phenols were categorized. Table 6-3 summarizes the rates of formation of these metabolites by tumor, liver, kidney, and lung microsomes and presents comparable data in rodents. There was a very large variation in the human metabolism of BaP, compared with that demonstrated in studies carried out concurrently in rodents. That might reflect, as the authors noted, either the controlled environment of the animals studied or a genetic variation in humans. In the human liver, activities for metabolite formation were substantially lower than those in rat microsomal fractions, and there were significant differences in the BaP-metabolite profiles. A greater proportion of benzene-ring metabolites was formed by human lung microsomes than by human liver and kidney microsomes (or rodent lung microsomes). The relative increase in the 9,10-dihydrodiol product, as well as some increase in the 7,8-dihydrodiol metabolite, accounted for the larger portion of this difference among lung, liver, and kidney microsomes. There is an apparent biologic inconsistency in these findings: although the human lung is the principal site of PAH tumorigenesis, as the authors observed, the 9,10-dihydrodiol product is suggested to have little biologic activity on further metabolism, whereas other tissues, such as the liver, formed large concentrations of the 7,8-dihydrodiol, a "proximate carcinogen." Nevertheless, the findings are important, providing not only data on comparative rates of formation of metabolites constituting the "HPLC profiles" in man and rodents, but also intertissue metabolic profiles of BaP biotransformation for three major organ systems in man. Thus, they extended the earlier findings of Selkirk *et al*.[160] on liver cells and lymphocytes.

A major advance in defining the role of the liver in PAH metabolism and the factors that regulate liver monooxygenase activity has been the demonstration that hepatic microsomal oxidation of specific substrates can be directly increased in vitro, in addition to their property of being induced in vivo, by various chemical treatments.[27,28,73,174] Conney and colleagues have shown that 7,8-benzoflavone added to homogenates of human liver samples can increase the rate of BaP hydroxylation by a factor of up to 11. Benzoflavone also increased some drug hydroxylations substantially, although benzoflavone at concentrations lower by a factor of about 100 paradoxically inhibited these reactions. Marked individuality for activating and inhibiting effects of benzoflavone were noted, and no significant effects on the oxidation of some drug substrates (e.g., coumarin and hexobarbital) were observed.

The enhancing effect of 7,8-benzoflavone on BaP oxidation was shown to extend to other flavonoids, such as flavone, nobiletin, and tangeritin. Related compounds--such as apigenin, chrysin, fisetin, flavonone, galargin, hesperitin, kaempferal, morin, myricitin, naringerin, and quercetin--inhibited BaP oxidation. The stimulatory effect of 7,8-benzoflavone on BaP 9,10-dihydrodiol oxidation to bay-region epoxides was also studied and shown to have significant species-specific characteristics. With untreated hamster microsomes, more than 60% of the total metabolites of the hydrocarbon were bay-region diol-epoxides, whereas human liver formed less than 5% of such metabolites. Addition of 7,8-benzoflavone to the microsomal incubations dramatically stimulated the formation of these metabolites in human (and rabbit) liver microsomes. The stimulatory effects of flavonoids on hydrocarbon oxidation have recently been shown to occur in vivo as well,[100] so the biologic importance of this phenomenon for the intact host is likely to be considerable. The mechanism of flavonoid activation of BaP hydroxylation has recently been explored in detail by Huang *et al*.[73] The flavone stimulates the NADPH reduction of cytochrome P-450, but not that of cytochrome c, by NADPH-cytochrome c reductase; this finding supports the idea that the catalytic sites for these substrates of the reductase are different. Other evidence that these catalytic sites are different has also recently been provided by the studies of Yoshinaga *et al*.[197-200]

Metabolic transformation of PAHs and their binding to cellular macromolecules in cultured human gut tissues have been described. Harris *et al*.[68] examined the metabolic fate of BaP and several other compounds in cultured esophageal explants from eight patients, six of whom had esophageal carcinomas. Metabolism of the ^{3}H-labeled PAH to water-soluble metabolites varied among the eight patients over the range of 1-68% of total metabolism; the variation found within a single case, however, in relation to different anatomic segments of the esophagus (proximal, middle, and distal) was quite narrow--2%. In spite of the 68-fold variation in metabolism among subjects, the patterns of conjugates formed from metabolites in general were qualitatively similar: sulfate esters, 21-55%; glucuronide conjugates, 7-37%; and glutathione conjugates, 24-66%. Most of the radioactivity of the organic-solvent-soluble metabolites of BaP cochromatographed with authentic metabolites of this compound, including its proximate carcinogenic, (-)-*trans*-7,8-dihydro-7,8-dihydroxy, derivative. Despite the predominant occurrence of esophageal cancer in the distal segment, the patterns of metabolites formed in all segments of the organ were similar. Binding of ^{3}H-labeled PAHs to DNA and protein was detected in all eight cases, with binding to protein greater than to DNA in each instance. Binding among the eight cases varied 99-fold, and at least three hydrocarbon-DNA adducts, including the specific guanine adduct, were recognized. The adducts appeared identical with those previously found in human colon and bronchus; and the patterns of both metabolism and adduct formation with BaP were analogous to those found in experimental animals susceptible to the carcinogenic action of PAHs.

Autrup et al.,[9] in an earlier and less detailed but essentially similar study, had reported comparable data based on human colon explants of tumor-free tissue. The binding of labeled BaP to cellular protein was several times higher than that to DNA; however, hydrocarbon binding to DNA correlated with tissue AHH activity ($r = 0.87$), whereas no such correlation existed for protein binding. DNA binding (BaP, picomoles bound per 10 mg) among seven tissue samples varied over a 25-fold range; variation within subjects was small. In an extension of this work, Autrup et al.[11] studied the comparative metabolism of BaP (and aflatoxin B1) and hydrocarbon-DNA adduct formation in cultured human and rat colon explants. Adduct formation (in 103 cases) varied over a 125-fold range in the tumor samples and in the same subject over a 3- to 10-fold range in different segments of the organ. A number of hydrocarbon-DNA adducts were identified, and both qualitative and quantitative rat-human differences were demonstrated. Although overall BaP metabolism was similar in rat and human colon tissue, the ratio of organic-solvent-soluble to water-soluble metabolites was higher in the human; sulfate esters predominated in rat colon, whereas equivalent quantities of sulfate esters and glutathione conjugates were formed in the human tissue; and hydrocarbon-DNA binding was distinctly greater in human colon, although, as noted, there was marked variability in adduct formation within a given subject.

The comparative hydration of styrene 7,8-oxide, octene 1,2-oxide, naphthalene 1,2-oxide, phenanthrene 9,10-oxide, benz[a]anthracene 5,6-oxide, 3-MC 11,12-oxide, dibenz[ah]anthracene 5,6-oxide, and BaP 4,5-, 7,8-, 9,10-, and 11,12-oxides to their dihydrodiols was investigated in microsomes from nine human liver samples obtained at autopsy.[80] The substrate specificity of the epoxide hydratase in human liver microsomes was very similar to that of the epoxide hydratase in rat liver microsomes. Phenanthrene 9,10-oxide was the best substrate for the human and rat epoxide hydratases, and dibenz[ah]anthracene 5,6-oxide and BaP 11,12-oxide were the poorest substrates. Plotting epoxide hydratase activity obtained with one substrate against epoxide hydratase activity for another substrate for each of the nine human livers revealed excellent correlations for all combinations of the 11 substrates studied ($r = 0.87-0.99$). The data suggest the presence in human liver of a single epoxide hydratase with broad substrate specificity, although the results do not exclude the possible presence in human liver of several epoxide hydratases that are under similar regulatory control.

CIRCULATORY SYSTEM

Juchau et al.[76,77] summarized a body of literature bearing on the hypothesis that PAHs may play an important role in the pathogenesis of arteriosclerotic lesions. The validity of this hypothesis apart, these investigators clearly demonstrated that aortic tissues from a number of species, including man, have detectable, albeit low, monooxygenase activities using BaP and 7,12-DMBA as substrates. Enzyme activities were comparable with those characterizing mouse skin. Cytochrome P-450 could be detected in primate aortas, and epoxide hydratase activity for BaP 4,5-oxide was identified in homogenates of the arterial walls of chickens and rabbits. The characteristics of the aortic monooxygenase for BaP resembled those of the enzyme system found in other tissues. It could be markedly induced, for example, by 3-MC, polychlorinated biphenyls (PCBs), and 5,6-benzoflavone; and, surprisingly, aortic homogenates produced higher than expected quantities (by as much as a factor of 28) of alkali-extractable metabolites when hematin was added to the reaction mixtures. Interestingly, hematin has been shown in other studies to degrade, in vitro, components of the monooxygenase system.[111] The primary BaP metabolites formed in rabbit aortic homogenates were the 3-OH and 9-OH derivatives, phenolic compounds known to be cytotoxic. The authors cited unpublished data to show that the aortic metabolites of BaP form covalent bonds with such macromolecules as calf-thymus DNA. Treatment of chickens with the inducer 3-MC markedly increased the amount of the PAH-DNA adducts, whereas addition of 7,8-benzoflavone in vitro inhibited binding. Aortic enzymes also have been shown to catalyze the formation of mutagenic metabolites from 7,12-DMBA. Thus, both cytotoxic and mutagenic metabolites of PAHs can be generated in vascular tissues. The possible relation of the formation of these compounds to the initial vascular injury that may presage the local development of an atherosclerotic plaque is of considerable interest.

The interaction of benz[a]anthracene and BaP with crystalline human serum albumin in solution has been studied fluorimetrically by Ma et al.[110] Equilibrium studies indicated that both PAHs bind to the protein to the same extent. Evidence of energy transfer from the tryptophan residue of the protein (increase in the weak B region--395-420 nm--fluorescence of the PAHs) permitted an assessment of the mean distance between the tryptophan and the bound ligand, thus identifying two different binding sites in the same general area. The authors suggested that structural differences among hydrocarbons, which may greatly affect their orientations on the protein molecule, influence mainly the selection of the binding site, rather than the binding equilibrium.

In vivo BaP associates very little with serum albumin in the presence of lipoproteins. The kinetics of BaP transfer between human plasma lipoproteins have been examined by Smith and Doody[163] with high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL) prepared from fresh unfrozen human plasma by ultracentrifugal flotation. BaP-lipoprotein interactions were analyzed fluorimetrically, and kinetic measurements were determined by stopped-flow techniques. The half-times of BaP transfer between HDLs, between LDLs, and between VLDLs were 40, 180, and 390 ms, respectively. The transfer of these PAHs among lipoproteins of the same density class was about one-twentieth that of pyrene under the same conditions. The rate of BaP transfer between lipoproteins also decreased with increasing size of lipoprotein; at equilibrium in vitro, VLDLs contain about 10 times more of the BaP than LDLs, and LDLs contain 20-50 times more than HDLs. The distribution between plasma and erythrocytes is different for 7,12-DMBA, BaP, benzanthracene, and anthracene, the mass of the PAH being associated with red cells (50, 70, 93, and 100%, respectively). Plasma lipid concentrations and the dynamics of lipid and lipoprotein metabolism clearly may have an impact on PAH distribution in blood and into specific tissues. For example, transfer of BaP is quite rapid, compared with the half-time for either hydrolysis of chylomicron triglyceride (about 2-5 min in humans) or clearance of the most abundant lipoproteins from the circulation (3-5 d in humans). The data of Smith and Doody[163] concerning the role of plasma lipoprotins in the transport of PAHs corroborated and extended the findings of other investigators who examined the interaction of PAHs and plasma proteins.[13,31,32,57,162]

The specific process of BaP uptake from human LDLs into cultured human cells was examined by Remsen and Shireman.[149] The cell lines used were WI-38, a human embryonic lung-fibroblast line, and GM 1915, a skin-fibroblast line derived from a patient with homozygous familial hypercholesterolemia; the former cells are LDL-receptor-positive, and the latter LDL-receptor-negative. Thus, in these studies, it was possible to explore the role of LDL receptors in the cellular uptake of PAHs that enter the bloodstream transported by chylomicrons and plasma lipoproteins. The results indicated that cellular uptake of the tritiated PAH by both cell lines from delipidated or serum-free medium varied linearly with concentration, whereas incorporation of PAH bound to LDLs was much less and, at higher lipoprotein concentrations, varied nonlinearly. The presence of the PAH in the LDL preparation did not affect the binding of ^{125}I-labeled lipoprotein to receptor-positive cells. The study provided several findings of special importance relative to the biologic impact of PAHs--or at least BaP as a model compound--on tissues in vivo. Clearly, although LDLs carry substantial amounts of PAH, the presence of LDL receptors on cells is not necessary for tissue uptake. The fact that PAH bound to LDL was incorporated into cells more slowly than PAH in a delipidated serum or serum-free medium raises questions about the biologic significance of experimental models in which increased incorporation of BaP from particles into lipid vesicles has been demonstrated. The data from these experiments also indicate that cells that may be directly exposed to a PAH (i.e., tracheobronchial, intestinal, and cutaneous cells) before the compound reaches the bloodstream may accumulate PAH in much higher concentrations than cells exposed to the PAH bound to lipoproteins, inasmuch as the latter significantly slowed as well as limited the cellular uptake of BaP. Finally, the report indicated that BaP previously incorporated into WI-38 cells could be substantially removed (by 55-79%) in a 120-min posttreatment study period by 10% delipidated serum or LDL-containing medium. This finding implies a potential for considerable PAH redistribution and a requirement for a not insignificant period for progression of the hydrocarbon from the plasma membrane to the endoplasmic reticulum, where metabolism takes place.

The ability of human monocytes to oxidize BaP and the induction of this enzyme activity by benzanthracene have been demonstrated by several investigators.[16,17,96,145] Lake and colleagues[96] re-examined this problem with the goal of developing a practical assay for measuring whole-cell metabolism of BaP under highly standardized conditions, eliminating--among other problems--the need for a large volume of blood (50 ml) in the fluorometric assay developed earlier for AHH activitity in this cell type. By measuring whole-cell generation of water-soluble BaP metabolites over a 3-d culture period, using ^{3}H-labeled substrate and closely controlling other characteristics, they provided a useful alternative cell system to that using mitogen-stimulated lymphocytes for characterizing BaP oxidation activity in humans.

Because of the advantage gained by the much greater inducibility of AHH activity (up to 40-fold) in cultured monocytes, compared with mitogen-stimulated lymphocytes (about 5-fold), the monocyte system was used by Okuda *et al*.[131] to study the contribution of genetic factors to the control of individual variation in AHH inducibility. Ten sets of monozygotic tissues were assayed two to four times and 17 sets of dizygotic tissues one to three times for basal and induced monocyte AHH activity. The results indicated that 55-70% of the individual variation in AHH inducibility of monocytes was genetically determined. Variation in AHH inducibility within subjects in repeat assays was wide and approached the magnitude of the variation between subjects. Thus, a single AHH assay is an imprecise biochemical characterization of a subject. Alternatively expressed, the method then available (late 1977) made it impractical to characterize a population with genetically distinct differences in AHH inducibility. The large intrasubject variation in AHH inducibility of monocytes also indicated that, in addition to the clear genetic influences on this process, unknown environmental or technical factors expressed themselves in the test procedure.

An abundant literature exists related to the monooxygenase activity of lymphocytes; the inducibility of this activity by mitogens, which have the property of stimulating lymphocyte transformation, during which a number of metabolic activities are concurrently greatly increased; and the use of mitogen-stimulated lymphocytes to study the genetic control of AHH in man and its relation to the occurrence of some human cancers--notably those of the lung. Kouri and colleagues have reviewed key aspects of this subject;[91,92] McLemore *et al*.[119-122] have also provided a detailed analysis of the genetics of AHH and its purported relation to human cancer. Only a brief summary of these findings can be included here.

The identification of AHH activity in lymphocytes in 1972[29,192] and its increase during lymphocyte blastogenesis led quickly to clinical studies, the earliest being that of Kellermann *et al.*,[87] in which this induced enzyme activity was measured in cultured lymphocytes of normal controls, non-lung-tumor controls, and lung-cancer patients. In a preceding study in the same year, this group[86] had examined the genetic variation in AHH activity in lymphocytes of 353 normal subjects and had categorized the population into three groups--low, intermediate, and high responders with respect to AHH inducibility; the population frequencies were about 50%, 40%, and 10%, respectively. The conclusion was reached that the enzyme activity was controlled by two alleles at a single gene locus and that the high and low responders were homozygous and the intermediate group heterozygous for those alleles. In the initial lung-cancer study,[87] there was a virtual absence of cases in the low-inducibility population, and all but two cases were in the intermediate- and high-inducibility categories. All the lung-cancer cases were in heavy smokers; of the 50 subjects, 48 had an average consumption of two packs of cigarettes per day. When the two control groups (normal subjects and a non-lung-cancer tumor group) and the lung-cancer group were compared for risk of lung cancer, those with intermediate and high inducibility (48 of the 50 lung-cancer cases) had risks for lung cancer 16 and 36 times, respectively, the risk in the low-inducibility group. This study prompted considerable controversy over the next few years, during which the findings of Kellermann and associates were cast in doubt.

A strong correlation (r = 0.923) was also found by Kellermann *et al.*[85] between the plasma elimination rate of antipyrine and the rate of BaP metabolism in human lymphocytes from a "carefully selected homogeneous" population, compared with the much lower correlation (r = 0.425) found in a "heterogeneous" population. The authors interpreted their findings as supporting the existence of common oxidative systems or common genetic control of the systems for antipyrine and BaP oxidation. Atlas *et al.*[7] confirmed that plasma antipyrine half-life is correlated to some extent with AHH inducibility (r = 0.84), although no intrasubject correlations were found between AHH inducibility and the oxidation of other drug substrates, such as phenylbutazone and bishydroxycoumarin. Most importantly, this group,[7] while affirming a significant heritable determinant of AHH inducibility in human lymphocytes, failed to confirm the monogenic model and trimodal distribution of AHH inducibility in the general population, proposed by Kellermann *et al.*;[86] rather, the population distributions for AHH inducibility (and for plasma antipyrine half-life) were consistent with polygenic control of both traits in man. In other studies in which the relation of AHH inducibility to the occurrence of lung cancer was re-examined by Paigen *et al.*,[133,134] low AHH activity was found in half the tumor patients studied, in contrast with the earlier findings of Kellermann *et al.*,[87] and no characteristic alterations in this enzyme activity were found in the progeny of these patients. A considerable number of technical problems related to the lymphocyte-AHH assay may confound the results obtained in studies of this enzyme activity and its relation to human cancer, as noted by Kouri et al.[91] However, recent methodologic advances made by this group, particularly the use of cryopreserved lymphocytes and close control of a number of assay variables, have added an important degree of precision to the assay.

Chrysene, one of several PAH derivatives (benzanthracene is another), has been shown by Snodgrass et al.[164] to induce AHH activity in cultured human lymphocytes (from normal subjects) with BaP as substrate. The individual variation in the monooxygenase activity observed with other inducers was also seen with chrysene.

The comparative metabolism of BaP in human lymphocytes and human liver microsomes has been studied by Selkirk et al.,[160] who examined the nature of the metabolites formed by each cellular system. The patterns of metabolites formed in both cell systems had characteristics quite similar to each other, with some exceptions--for example, among the derivatives formed in a 30-min incubation, all three dihydrodiols produced by liver were absent in the lymphocyte incubation mixture. In a 24-h incubation of lymphocytes, however, all three dihydrodiols formed by liver microsomes were also formed by the blood cells, and new metabolite peaks were observed, presumably reflecting more extensive biotransformation of already formed metabolites in the reaction mixture. The authors concluded that, although the ratios of some metabolites may differ and although lymphocytes form several more derivatives than does liver, many identical metabolites are produced in these two human cell types.

Schönwald et al.[158] studied the effect of BaP on sister chromatid exchange in mitogen-stimulated lymphocytes of 11 normal subjects and 18 patients with lung cancer. Patients and controls differed neither with respect to the spontaneous rate of sister chromatid exchange nor in their responses to the hydrocarbon, although it did double the number of exchanges in both population groups.

Barfknecht et al.[15] studied the ability of dichloromethane extracts of automobile diesel soot at high concentrations (100 mg/m^3) to induce trifluorothymidine-resistant mutants in human lymphocytes incubated in the presence of rat-liver postmitochondrial supernatant. A significant induction of such mutants was observed. Anthracene, phenanthrene, and their alkylated derivatives accounted for one-fourth of the observed biologic activity. Among eight related compounds, there was general agreement between responses in lymphoblasts and in bacterial test systems. Phenanthrene was an exception, in that it was positive in the human-lymphoblast test system, but negative in bacteria at a concentration 60 times higher. The data in this report indicate that methyl substitution at some sites of anthracene and phenanthrene greatly increases their mutagenicity in both S. typhimurium and human lymphoblasts. A similar effect for chrysene has been observed. Methylations at the 1 and 3 positions of phenanthrene and the 2 and 9 positions of anthracene result in PAHs that are particularly mutagenic in the human and bacterial test systems used. Methylations at other positions had the capability of eliminating the mutagenic activity of the PAH derivative. No correlation between the results of the mutagenesis studies with the soot-derived PAHs and the reported capacity of the compounds studied to elicit neoplastic or carcinogenic responses in test animals could be made.

REPRODUCTION

The title of this section refers collectively to studies related to the ability of some genital tissues (including the placenta) to metabolize or otherwise respond biochemically to PAHs. There is an abundant and detailed literature on transplacental[151] and perinatal[182] carcinogenesis. These and related topics in reproduction were reviewed in a 1981 special issue of the Journal of Environmental Pathology and Toxicology[154] and are not summarized here. It is perhaps appropriate, however, to refer to the report by Sir Percival Pott in 1775,[142] in which there was first described an increased incidence of scrotal cancer in chimney sweeps exposed to soot, and to note that almost 150 yr elapsed before Yamagiwa and Ichikawa[194] demonstrated that the repetitive application of crude coal tar to the rabbit ear produced skin cancer and that the identification of specific carcinogenic coal-tar constituents, such as BaP, required the passage of additional decades.[20,43,88] Over this period, the question of why only scrotal cancers, and not other genital cancers or even other cancers in general, were found in excess in chimney sweeps appears to have remained unanswered.

Grover *et al*.[66] investigated the metabolism--including the specific identification of biotransformation products--of three ^{3}H-labeled PAHs by nonneoplastic human mammary epithelial-cell aggregates maintained in culture. The lobuloalveolar units from which these aggregates are derived are thought to be the site of origin of many human mammary carcinomas; two of the PAHs studied, 7,12-DMBA and BaP, are known to be relatively potent mammary carcinogens in rats, whereas benz[a]anthracene is not a mammary carcinogen in rats. Tissues from eight patients were studied. The extent of metabolism of the PAHs is summarized in Table 6-4. There was considerable individual variation in PAH metabolism among the subjects studied, but the formation of water-soluble metabolites by the tissue samples accounted, in each instance, for a major portion of the total of each PAH metabolized. The extent of binding of each PAH to cellular DNA and proteins also varied considerably. Interestingly, the extent to which ^{3}H-labeled metabolites of benz[a]anthracene--a noncarcinogen for mammary tissue in the rat--were bound seemed, from the limited data obtained, to be consistently lower than the binding displayed by the other two PAHs. The results of chromatographic characterization of PAH-DNA adducts formed suggested that, with BaP, the hydrocarbon was activated by the cultured cells through the formation of *anti*-BaP 7,8-diol-9,10-oxide, a bay-region diol-epoxide that appears to be responsible for most of the nucleic acid adducts formed in several other biologic systems. The situation was less clear with 7,12-DMBA, although a portion of the adducts formed with this PAH cochromatographed with adducts present in a DNA hydrolysate that had been treated with anti-7,12-DMBA 3,4-diol-1,2-oxide--a derivative that is also classified as a bay-region diol-epoxide. The authors interpreted their data with caution, considering all the factors known to bear on the development of mammary cancer; but the possibility of partial causal relationships among the PAHs, their metabolic transformations, and tumor stimulation is implicit in this work.

Stampfer and colleagues[168] did similar studies with BaP and cultured mammary epithelial cells and fibroblasts. They showed that the breast epithelial cells were 50-100 times more sensitive (growth inhibition) to BaP than the fibroblasts; that the epithelial cells formed adducts as early as 6 h after addition of the PAH to the cultures; and that the adducts between the 7R anti stereoisomer of BaP diol-epoxide and deoxyguanosine predominated at all times and, with two minor adducts that were consistently present, persisted in the epithelial cells for at least 72 h in a BaP-free medium. No adducts were detected in fibroblasts until 96 h after exposure to the PAH, at which time the type and extent of adduct formation were similar to those observed with epithelial cells. As with the report of Grover et al.,[66] caution concerning the direct relation of these findings to the role of PAHs in mammary carcinogenesis is necessary. On this matter, Stampfer and co-workers[168] stated, however, that "chemical carcinogens, particularly BaP, should not be minimized as possible factors in the initiation of breast cancer."

Mass et al.[113] studied 26 specimens of normal human endometrium to determine the patterns of metabolism of [^{3}H]BaP in short-term explant cultures. Three of the tissue samples were from postmenopausal women; of the remaining 23, it was possible to approximate the stage of the menstrual cycle at which the tissue was removed during surgery. Eight of the latter subjects were smokers. In summary, it was clear that normal human endometrium could enzymatically convert BaP to a wide variety of oxygenated derivatives that cochromatographed with dihydrodiols, quinones, and monohydroxy products of the PAH; sulfation was also identified. HPLC analysis of metabolites revealed marked individual variation in metabolite formation among the subjects studied; smoking did not account for this difference, but some evidence of hormonal influences on the patterns of PAH metabolism was adduced.

In a study by Dorman et al.,[52] BaP binding to DNA in human endometrial tissue was studied in samples obtained from 41 subjects and, again, a striking (70-fold) range in the observed specific activities of carcinogen binding to DNA was identified (see Figure 6-1). Tissues obtained late in the proliferative phase or early in the secretory phase of the menstrual cycle had the highest mean specific activity of PAH-DNA binding (Table 6-5). Binding was significantly reduced when tissue specimens from low-estrogen periods of the menstrual cycle were studied. The reason for this apparent association between estrogen content (actually, the estimated phase of the cycle) and PAH-DNA binding is obscure, but clearly merits further study. Such study would have to deal with the important confounding factor of the broad range of individual variation in binding, which may mask systematic but small changes that can occur during a menstrual cycle, but which cannot now be detected.

Namkung and Juchau[128] studied the oxidative biotransformation of BaP in preparations of human placental microsomes with HPLC. The investigations revealed that the use of substrate concentrations high enough to ensure zero-order reaction kinetics markedly inhibited the formation of dihydrodiols in the reaction mixtures. The relative quantities of dihydrodiols generated increased with decreasing substrate concentrations between 200 and 2.7 μM. Addition of manganese or ferric ions to reaction mixtures altered the ratios of generated phenols to dihydrodiols. Identical results were obtained with ^{14}C- and ^{3}H-labeled BaP as substrate. The data suggested that considerable amounts of 7,8-dihydroxy-7,8-dihydro-BaP, a proximate mutagen-carcinogen, may be generated in vivo by placental tissues of women who smoke.

The formation of PAH metabolite-nucleoside adducts when human tumor placental microsomes were incubated with [^{3}H]BaP and salmon sperm DNA has been studied by Pelkonen and Saarni.[139] There were significant differences between the PAH metabolite patterns and the nucleoside-metabolite complexes formed, compared with rat liver, for example. Specifically, in the human placenta microsomes, the absence of the nucleoside complex of 9-hydroxy-4,5-oxide implied the inability of this tissue to form 4,5-oxides of BaP. Indirect evidence of epoxide hydratase activity in placental tissue was obtained. The extent of PAH-DNA binding in this tissue correlated significantly with both 7,8-diol metabolite formation and fluorometrically determined AHH activity. The question of whether the 7,8-diol-9,10-epoxide of BaP is formed by the human placenta in vivo could not be answered unequivocally, but the authors' inferential conclusion is that it is probably formed in the human host. The interplay of possible genetic influences and clearly established regulatory influences of environmental factors on human placental AHH has been incisively discussed by the same group.[138]

Cigarette-smoking has been shown by Conney and associates[42,189,190] to be one of the most potent and consistent inducers of human placental AHH activity yet identified. In the initial report of the group,[189] the enzymatic hydroxylation of BaP could not be detected in nonsmokers in homogenates of placentas frozen immediately after birth and studied within 48 h. In contrast, the enzyme activity was present in all 11 placentas from women who smoked during gestation, although enzyme activity in this small group did not correlate with the number of cigarettes smoked. BaP administration to pregnant rats also was shown to induce AHH activity in the placenta. The effect was related to PAH dose. This study constituted the first demonstration that compounds in cigarette smoke could induce a carcinogen-metabolizing enzyme in human tissues. These studies were extended[190] to related enzymatic reactions in human placentas and to other types of pyrenes as probes for AHH-inducing activity in rat placenta (see Table 6-6). Extremely active inducers included chrysene, 1,2-benzanthracene, pyrene, 3,4-benzofluorene, and a number of related compounds.[190] The wide variability in the induction of AHH activity in human placentas is exemplified by the data in Table 6-7--a range in activity of the enzyme in smokers approaching 1,000-fold (a nearly 2,000-fold range if smokers are compared with nonsmokers). The basis for this extreme range of responses to a chemical exposure (15-20 cigarettes/d for each subject) is not known. However, data presented by Harris *et al.*[70a] suggests that pulmonary alveolar macrophages can metabolize BaP to proximate and ultimate mutagens released into extracellular space.

LUNG

The respiratory tract comprises an extremely disparate and complex set of tissues containing some 40 different cell types.[166] As Devereux *et al.*[47] have noted, whereas pulmonary cytochrome P-450 and the metabolism of xenobiotics have been studied with various preparations of lung tissue (microsomes, isolated perfused lung, cells obtained by pulmonary gavage, direct instillation of xenobiotics in various portions of the respiratory tract, etc.), little is known about the localization of the cytochrome P-450 monooxygenase components in the pulmonary system. This section deals exclusively with the metabolic properties of human respiratory tissues with respect to PAH metabolism, but the lack of information just cited needs to be kept in mind. There are facets of the investigation of Devereux *et al.*[47] in rabbits that probably bear significantly on problems of human pulmonary tissue biotransformations that depend on cytochrome P-450; these aspects include the observation that the alveolar macrophage that accumulates PAH has little or no measurable cytochrome P-450 or monooxygenase activity[58,71,148] and that there is selective cellular distribution of cytochrome P-450 species.

The ability of human bronchial epithelial cells to bind and presumably to activate such PAHs as 7,12-DMBA, 3-MC, BaP, and dibenz[ah]anthracene was described by Harris and colleagues in 1974.[70] Four tissue samples were studied (one control and three lung cancer) in explant cultures, and radiolabeled PAHs were used; radioactivity from all four compounds tested was found in both cytoplasm and nuclei and in all tissue samples studied (see Table 6-8). The number of tissues examined precluded comparisons between normal and tumorous lung PAH metabolism, and no studies of PAH-DNA adduct identification were carried out, although, as noted, radioactivity from the labeled PAHs was found tightly bound to DNA isolated by CsCl gradient. A more detailed study by this group[195] used tissues obtained from an additional four patients, three of whom had pulmonary malignancy.

Explants of human bronchi also metabolized BaP and released derivatives that are mitogenic in the Chinese hamster V-79 cell line.[72] The 7,8-diol of BaP was approximately 5 times more potent as a promutagen than the parent PAH; binding of the diol to DNA was 5-20 times greater than that found with BaP. When 13 samples of bronchial cells were studied with cloned Chinese hamster V-79-4A cells, a positive correlation between DNA-PAH binding (in the cultured bronchial cells) and induction of 0^r (ouabain-resistant) mutants was found, but no correlation between this mutation frequency and AHH activity was identified. This may be attributable, as the authors noted, to the difficulty in correlating AHH activity with the consequences of the multistep pathway of metabolic activation for BaP. The individual variation in mutation frequency was 9-fold, and the variation in binding of PAH to DNA 5-fold. This important investigation pointed the way toward study of the metabolic activation of chemical carcinogens into promutagens and mutagens directly in differentiated epithelial cells derived from human tissues; and the human tissue-mediated mutagen assay opened the possibility of testing the hypothesis that people differ in mutagenic and oncogenic susceptibility to environmental chemicals, depending on individual capacity to activate and deactivate chemical procarcinogens. Autrup _et al_.[12] compared the metabolism of BaP by cultured tracheobronchial tissues from humans and four other species (mice, hamsters, rats, and cows). They provided evidence that the metabolism of BaP is qualitatively similar in tracheobronchial tissues from humans and from animal species in which PAHs have been shown experimentally to be carcinogenic.

A similar study limited to a comparison of human lung microsomal fractions and rat microsomes was carried out by Prough _et al_.[144] The results indicated that human microsomes form a higher percentage of dihydrodiol products from BaP than do rat microsomes. The wide variation of PAH metabolite profiles formed by the 15 samples of human lung studied may be due in part to differences in clinical diagnosis when the samples were obtained. Bronchial tissues cultured in a chemically defined medium were exposed to radiolabeled BaP or its metabolites, and their binding to DNA was measured. Radiolabeled metabolites were prepared by incubating the parent PAH with rat liver microsomes and then purifying and identifying with silica gel and HPLC. The binding data showed that (-)-_trans_-7,8-diol bound to bronchial mucosal DNA to a considerably greater degree (5- to 23-fold) than did BaP; binding was also much greater (25- to 80-fold) than with the (-)-_trans_-9,10-diol. The _trans_-7,8-diol constituted 3-6% of the total identified metabolites when human bronchi were exposed to BaP. Diol-epoxides were formed from (-)-_trans_-7,8-diol in two of the bronchial explants, and strong evidence was provided that the major tumor bronchial mucosal DNA-binding BaP metabolite is in fact derived from (-)-_trans_-7,8-diol.[195] The specific adducts formed between DNA and the metabolic intermediates of BaP were not isolated, but the author concluded that the predominant bound metabolite is a single enantiomer of diol-epoxide I derived as indicated above.

In an extension of their earlier work, Harris and colleagues[69] examined the metabolism of BaP and 7,12-DMBA in explants of human bronchus and made a metabolic comparison with human pancreatic duct explants. As in the prior study, both normal and malignant human bronchi (37 subjects) metabolized BaP actively and in generally similar fashion, except for a higher percentage of organic-solvent-extractable metabolites formed by bronchi from noncancer patients. In addition, prior exposure of the bronchial explants to benz[a]anthracene altered the qualitative features of the metabolite profile of BaP, as analyzed by HPLC. Benz[a]anthracene specifically increased the binding of BaP to cellular DNA and the activity of AHH. Among a group of 28 of the patients' tissues studied, 7,12-DMBA was bound to DNA more often (26 of 28) than BaP. In the comparison with pancreatic duct explants, 7,12-DMBA-DNA binding was consistently lower in the latter tissue than in the bronchial explants.

Cohen *et al.*[34] showed, with cultured human bronchial epithelium, that BaP was converted promptly to metabolites that cochromatographed with 9,10-dihydro-9,10-dihydroxy-BaP and 7,8-dihydro-7,8-dihydroxy-BaP. Similar results were obtained with human lung cultures, except that a major metabolite, benzo[a]pyrene-3-yl hydrogen sulfate, was identified. The biologic activity of this sulfate ester of 3-hydroxy-BaP is of interest, because, owing to its physicochemical properties, it could be extremely persistent in man.

Covalent adducts between DNA and BaP in treated cultured explants of peripheral human lung tissue and in the continuous human alveolar tumor cell line were identified by Shinohara and Cerutti.[161] From the chromatographic analysis of digests of DNA extracted from these tissues, it was concluded that both the lung specimens and the human alveolar tumor (A549) cells metabolized BaP to diastereomeric 7,8-dihydroxy-9,10-epoxytetrahydro-BaP intermediates that mostly reacted with the exocyclic amino groups of deoxyguanosine to form N^2-(10-[$7\beta,8\alpha,9\alpha$- and 9β-trihydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene]yl)deoxyguanosine (dGua-BaP I and II). Although comparable amounts of dGua-BaP I and II were formed in A549 cells, dGua-BaP I was the predominant adduct in the DNA of lung specimens from six different donors.

The wide range of metabolic capacities for PAHs exhibited by other human tissues studied also extends to lung tissue, as shown by Cohen *et al.*[35] They observed a 44-fold variation in the ability of short-term organ cultures of peripheral lung tissues from human cancer patients to metabolize BaP to organic-solvent-soluble derivatives. The total amounts metabolized ranged from 1% to 96.2% in a 24-h culture period. The authors concluded that, although caution must be exercised in measuring metabolic activities of human tissues derived from diseased patients, the use of short-term organ explant cultures mimics the in vivo metabolic disposition of PAH better than the use of lymphocyte AHH activity would. A solution to the practical problem of obtaining lung tissue from large populations to study the validity of this conclusion is not apparent.

Kahng *et al.*[78] concluded from a study of 11 immediately autopsied subjects that bronchial tissue exposed to benz[a]anthracene produced induction responses of AHH that correlated with induced AHH activity in monocytes from the same subjects. A reconfirmation of the wide range of individual differences in AHH activity of surgically obtained specimens of normal lung tissue (86 subjects) came from a detailed study by Sabadie et al.[153] Briefly, AHH activity was lower than normal in tumorous lung sections in 73 of the 86 patients; and in 22 tumor tissue samples, no AHH activity was detected at all. Individual variation (excluding the 22 subjects) in lung-tumor AHH activity was 20-fold, which approximated the variation observed in other studies, including those in which PAH-DNA binding and pulmonary tract tissues were studied. BaP metabolite formation was analyzed, and the results generally conformed with the data of other investigators.

Interestingly, BaP (but not pyrene) induces AHH and prolyl hydroxylase activity in neonatal rat lungs in organ culture.[74] Because prolyl hydroxylase is an indicator of collagen synthesis and increased activity of this enzyme in lung reflects increased collagen formation, the authors, Hussain et al., hypothesized that the earliest events in BaP-induced lung injury may include alterations in collagen metabolism. In a study of the effect of tobacco-smoke compounds on the plasma membrane of cultured human lung fibroblasts, Thelestam et al.[175] examined 464 compounds, of which nearly one-fourth gave rise to severe membrane damage. PAHs proved inactive in this test system; the PAHs tested included anthracene, benz[a]anthracene, chrysene, pyrene, BaP, perylene, fluoranthene, and coronene. The significance of these findings is not entirely clear, but, inasmuch as very large concentrations of the compounds were used (25 mM), the failure of all PAHs tested to cause substantial release of the radiolabeled nucleotide material from the cells suggests that PAH entry into cells of organs in which their carcinogenic potential is expressed does not require as an initial event plasma membrane damage by the active chemical species.

Lung damage by ozone[59] and nitrates[183] showed contradictory effects: in the former case, adaptation may become apparent, and, in the latter, susceptibility to infection may increase. In the case of asbestos-produced damage, as well as damage produced by other particles--such as iron oxide, silica, and carbon black--cellular uptake and availability of BaP increase.[97-99] Asbestos, of the several particles tested, was particularly effective in increasing microsomal uptake of the PAH, although clearly adsorption of the PAH on the particles--rather than simple mixture of the two--is required for the increase in cellular uptake to become evident. The relevance of these findings to the phenomenon of particle-PAH cocarcinogenesis is clear.[99] BaP elution from typical soot from pollution sources, as well as from soot in lungs (11 cases), has been carefully studied by Falk et al.[56] Strikingly, this PAH could not be recovered from soot in human lungs without malignancy (Table 6-9), whereas the noncarcinogen pyrene could be identified (in much lower concentrations than expected). Adequate controls appeared to ensure that the disappearance of the carcinogenic PAH was a biologic phenomenon taking place in vivo; the authors concluded that elution must have occurred in the host through an undefined mechanism. In another study,[178] involving 21 bronchial carcinomas, a search was made for 12 PAHs in the tissues with chromatographic and fluorescence techniques. Only four of the 12 PAHs sought were found: BaP, fluoranthene, perylene, and benzo[b]fluoranthene (Table 6-10). BaP was found in all tumors; fluoranthene and benzo[b]fluoranthene were sometimes present, as was perylene. Coronene, dibenz[ah]anthracene, pyrene, benz[a]anthracene, chrysene, benzo[ghi]perylene, benzo[k]fluoranthene, and benzo[e]pyrene were, if present, below the limits of detection.

HUMAN EXPOSURES TO PAHs: A BRIEF SUMMARY

The studies reviewed in the preceding sections were related primarily to the metabolic interactions of PAHs and human tissues and focused principally on the oxidative reactions known to convert many of these compounds to potent mutagens and carcinogens. This section reviews a number of reports dealing with possible detrimental health effects of specific workplace exposure to PAHs and representative reports dealing with PAH contamination of the aquatic environment and of foods. The literature on atmospheric exposure to PAHs is dealt with elsewhere, except for exposures that are discrete and intense, as in some working environments. In the light of this review, one cannot avoid the conclusion that the greatest present source of human PAH exposure is through the gastrointestinal tract; nor can one disagree with the statement in the 1970 Royal College of Physicians report[152] that, to the extent that PAHs are involved in the genesis of pulmonary malignancies, "by far the most important matter affecting all . . . aspects of mortality from lung cancer is smoking." The equally emphatic conclusion of Pike and Henderson[141] that "the epidemiologic evidence implicating cigarette smoking as the major cause of lung cancer is overwhelming" puts the clinical studies reviewed here related to the potential pulmonary hazards of atmospheric PAHs in proper perspective.

WORKPLACE EXPOSURE

Schenker in 1980[155] reviewed the question of whether diesel exhaust is an occupational carcinogen and summarized a number of the principal studies (Table 6-11) on the question of cancer incidence in populations of workers exposed to diesel exhaust. Data on environmental and occupational BaP and total suspended particles in various urban and rural sites and specific occupations were also provided (Table 6-12). These epidemiologic data emphasize the conclusion that "the carcinogenicity of workplace exposure to diesel engine exhaust is suggested . . . but the existing data are sparse and contradictory." Table 6-11 shows only concentrations of BaP, and the values are in units of micrograms per 1,000 cubic meters. Because the air breathed by a normal adult approximates 15-20 m^3/d, the highest PAH concentration shown indicates a potential exposure dose of about 700 µg/d in a work setting (coal and pitch-coking plant) known to have one of the most intense PAH exposures. This figure exceeds by orders of magnitude the exposure produced by the heaviest smoking, and such an occupational locale would thus be expected to elicit detrimental and clearly detectable health effects in man. The same consideration applies to the data on workers in gasworks retort houses and roof tarrers. But beyond these specific occupational sites, the respiratory intake of BaP--even if, for occupational purposes, a person had to remain for 24 h/d in Blackwall Tunnel, London (Table 6-11)--would approximate that from about a pack of old-style cigarettes per day. The improbability of such occupational exposures emphasizes the difficulty of measuring the health hazards of atmospheric PAH sources in the general sense (i.e., in the 28 rural and 24 urban sites depicted in Table 6-11).

A number of occupational-epidemiologic studies have emphasized the difficulties of reaching firm conclusions with respect to the direct (or measurable) health risks of PAHs in work environments, whether the suspected hydrocarbon comes from diesel or other automotive exhausts or from chemicals, such as petroleum sources, that are intrinsic in the occupation itself. Battigelli et al.[18] studied 210 locomotive repairmen (average age, 50 yr; average work period, 10 yr) considered to be regularly exposed to diesel exhaust and 154 "control" railroad workers. The studies were carried out in two railroad shops in Pittsburgh, Pa. The clinical data were scanty, and it was not possible to differentiate the exposed from the nonexposed worker population on the basis of pulmonary-function tests. However, smoking clearly impaired the pulmonary functional performance of workers. A somewhat comparable environmental study carried out by El Batawi and Noweir[55] in two diesel-bus garages in Egypt raised the possibility of clinically detrimental, synergistic effects of smoke and acrolein gas, which is known to be present in exhaust of diesel engines. Ventilatory-function changes over a workshift in coal miners exposed to diesel emission were studied by Reger et al.;[147] the only positive finding in this study of 800 men was that smokers suffered consistently greater pulmonary-function decrements over a workshift than nonsmokers. In a retrospective study of mortality statistics,[83] Kaplan could identify no higher than normal rates of death from bronchopulmonary carcinoma in workers exposed to fumes from diesel engines among the medical records of 6,500 deceased railroad workers, including 818 deaths from malignant diseases.

Lloyd et al.[109] reported that the mortality from respiratory cancer for men employed in a coke plant was twice the rate generally observed among steelworkers; the whole difference was accounted for by a threefold excess for nonwhite workers. A more detailed analysis[108] showed the following: The excess of respiratory cancer previously reported for coke-plant workers was limited to men employed at the coke ovens, the relative mortality for this disease being 2.5 times that predicted. The greatest part of the excess was accounted for by an almost fivefold risk of lung cancer in men working on the tops of the coke ovens. A 10-fold risk of lung cancer was observed for men employed 5 yr or more at full-time topside jobs; 15 lung-cancer deaths were observed among the 132 men in the topside group, compared with 1.5 expected. The apparent differential in respiratory-cancer rates for white and nonwhite coke-plant workers reported in an earlier paper was accounted for by differing distributions by work area and the unusually high lung-cancer risk for topside workers; lung-cancer mortalities for white and nonwhite coke-plant workers employed at work stations other than topside were comparable. A deficit of deaths from heart disease, previously reported for similar occupational groups, was also seen for coke-oven workers. Coke-plant workers employed only in nonoven areas may be at excess risk of digestive cancer.

A review of the literature on cancer mortality of men employed in the coal-tar industries showed that all these occupations evidence excess cancer at one or more sites. The lung-cancer excess in coke-oven workers also was observed in other groups engaged in coal carbonization, and it appeared that the lung-cancer response was positively correlated with the temperature of carbonization.

Among coke-oven workers studied by Mazumdar *et al.*,[117] excessive deaths from respiratory malignancy were reported. As in the study of Lloyd *et al.*,[109] there was a tendency for the death rates of nonwhite workers to be higher than those of white workers. Measured concentrations of coal-tar pitch volatiles in the environment of men who worked at the top of coke ovens were 2-3 times higher than in that of men employed at the side of the ovens. High BaP emission, among others, has been measured in the gaseous discharge--including the coal-tar pitch volatiles--of coke ovens in the steel industry, a rough estimate being that 1.8 g of this chemical is emitted per ton of coke produced.[117] As in the Lloyd *et al.* study,[109] the overall cancer-death risk for coke-oven workers was distinctly higher than that for normal persons in the age group over 55 yr, and the age-adjusted death rates for lung cancer showed a strong relationship between extent of exposure to coal-tar pitch volatiles and lung-cancer mortality. The lowest-exposure group[117] had death rates similar to those of nonoven workers, but all higher-exposure groups had age-adjusted rates that ranged from 3 to 10 times those of the comparison group with increasing exposure. The data in this study also confirmed the long latency in cancer formation, even under the conditions of high exposure to carcinogens characterizing coke-oven workers; the time between first exposure to coal-tar pitch volatiles and death from lung cancer varied from 10 to 40 yr, with an average of 25 yr.

Toxicologic experience with workers in the developing shale-oil industry is incomplete, although historical evidence indicates that potential health hazards related to malignancy may exist in the processes involved in oil extraction.[187] Some data on the content of BaP and pyrene analogues from shale materials, as reported by Weaver and Gibson,[187] are useful to record here (Tables 6-13 and 6-14). Because the industry is still in its developmental stage in this country, the overall health impact that may be attributed to exposure to these PAHs--as well as to other contaminants, such as arsenic, beryllium, cadmium, lead, mercury, and nickel[187]--is difficult to estimate.

EXPOSURE TO PAHs VIA THE GASTROINTESTINAL TRACT

The exposure of humans to PAHs may be considered to be almost exclusively via the respiratory and gastrointestinal tracts. Some occupational groups (e.g., the chimney sweeps studied by Pott) may have an intense local cutaneous exposure to PAHs, but the significance of percutaneous absorption of these compounds for the general population is not known. Such substances as the polychlorinated biphenyls[22] and constituents of coal tar[21] can pass through the skin and induce liver oxidative enzymes in animals, so it is possible for some (undoubtedly small) degree of PAH accumulation to occur in humans systemically via skin exposure.

Several major reviews of the importance of water and food as vehicles of human exposure to PAHs have been published in the last 5 yr. These include a special issue of the *Journal of Environmental Pathology and Toxicology*[154] devoted to the health aspects of PAHs and several monographs focusing on PAHs in drinking-water sources and on PAHs in the marine environment.[19,129,181]

PAHs in Water

It can be stated at the outset that human exposure to PAHs through the ingestion of water is quantitatively insignificant, compared with exposure through food--the contribution of drinking water is estimated to be only about 0.1% of the total PAH derived via the gastrointestinal tract in humans.[3] This estimate, carrying with it an implicit assumption of relative biologic safety (at least compared with foods as a source of PAHs), is probably valid except perhaps for some surface-water sources, which, because of location (e.g., downstream from shale-oil effluent or coke-byproduct discharge sites--see Table 5-12 of Santodonato *et al.*[154]), may be heavily contaminated by such PAHs as BaP. Groundwater concentrations of this prototype PAH determined in multiple German and American sources are extremely low (see Table 5-11 of Santodonato *et al.*[154]), ranging from a fraction of a nanogram per liter to several nanograms per liter. The average "total" PAH content is, of course, greater, but still in the same range. In contrast, low- to medium-concentration contaminated surface waters may contain PAHs 5-20 times higher, and this pollution may be increased by several orders of magnitude in sewage water or in surface waters adjacent to industrial sites. Treatment of surface water to obtain drinking water can nevertheless remove the bulk (95% or more) of the PAHs, particularly with activated-carbon filtration. This reflects the fact that much of the PAH in water subject to pollution is quickly adsorbed on suspended solids or is found in sedimented particulate matter. The majority of PAH entering surface water is concentrated locally; although PAH can probably be considered ubiquitous in water, the amounts involved are substantially lower than those found in air or on land. Neff[129] has pointed out that, if all PAHs found in the aqueous environment were distributed evenly throughout the oceans and fresh-water bodies, they would be undetectable and inconsequential.

As noted, the PAH content of drinking water is, with an occasional exception, low, as expressed as BaP and total PAH (Table 6-15).[154] Among the general class of PAHs, the compounds that have been detected by high-resolution gas chromatography after extraction from tapwater[132] are listed in Table 6-16 with their concentrations. Such contamination at a typical, most proximate (tapwater) drinking-water source represents only trace contamination, compared with the PAH content of original fresh-water sources, marine and estuarine waters, fresh-water and marine sediments, and some alcoholic beverages.[179]

The occurrence of PAHs in saltwater sources has for several reasons more potential biologic importance than the occurrence of these compounds in drinking water. The oceans provide a very large surface area for deposition of airborne PAHs via rain and dry fallout. Runoff of PAHs from the land surface also contributes substantially to marine-water content, as do direct effluents from sewage and industry. Carcinogenic PAHs occur in crude and, particularly, refined oils,[3] and oil spills may contribute in a major way to marine pollution with these compounds, especially on a local scale. The oceans constitute an ecosystem in which varied animal and plant life can participate in the metabolic processes involved in the uptake, storage, concentration, biotransformation, and discharge of PAHs. Thus, the consumption of fish and shellfish of predominantly saltwater, compared with fresh-water, origin (88% vs. 12% of the seafood in the diet) gives special importance to the PAH contamination of the aquatic environment that these food species inhabit.

PAHs are universally, although unevenly, distributed throughout the marine (saltwater) environment. They are derived principally from atmospheric fallout, terrestrial runoff, and spills of petroleum products. The contribution, if any, of marine organisms to PAH pollution by de novo biosynthesis is unknown. Total PAH entry into the marine environment from petroleum spills is estimated at 17 x 10^4 tons/yr, of which BaP would constitute 20-30 tons/yr.[129] Conservative figures for the total world contribution of industrial and domestic wastewaters to marine pollution with PAH have been estimated to be BaP at about 29 tons/yr and total PAH at 4.4 x 10^3 tons/yr. For terrestrial runoff, the figures are about 118 tons/yr and about 2.9 x 10^3 tons/yr, respectively, and for atmospheric fallout, 500 tons/yr and 50,000 tons/yr. Because the composition of total PAH in these sources varies considerably, it has been suggested[129] that the figures estimated for BaP input provide a better index of the potential carcinogen input from these sources than do the figures for total PAH.

The majority of PAH in the aquatic environment remains near the point source of contamination and thus is concentrated in coastal waters; here, the bulk of the PAH is in bottom sediments and to a lesser extent in suspended solids or solution. The water solubility of carcinogenic PAHs is very low, but solubilization may be increased by the concurrent presence of detergents and other organic substances. Photodegradation of PAHs in the marine environment can occur variably, depending on the depth and turbidity of water and other factors; but persistence of PAHs is much greater in water than in air, because the particulate matter on which these compounds are mostly adsorbed provides a storage pool from which they may be slowly returned to the water by leaching or through biologic processes involving marine organisms.

The characteristics of marine pollution by PAHs are such as to suggest the occurrence of multiple varieties of discrete ecosystems with relatively high concentrations of these compounds in sediments and local plant and animal species--all existing in a vastly larger aquatic environment characterized by a smaller degree of PAH contamination. In the local marine areas of high PAH pollution--principally river basins and estuarine and coastal waters--the degree of PAH contamination and the PAH composition in water, sediments, and nonmigratory marine life are determined by the nature of the point sources of contamination. In the organisms found in these areas, the PAH composition depends on metabolic processes related to the selective bioconcentration, biotransformation, and accumulation of the PAHs or metabolites or their discharge into the aquatic environment.

The fate of PAHs in marine ecosystems has been studied by Lee _et al._,[101] who used as a model Prudhoe crude oil enriched with a number of PAHs dispersed into a controlled ecosystem (polyethylene enclosure 2 m wide and 15 m deep) suspended in Sadnich Inlet, Canada. The oil was estimated to contribute PAHs at concentrations ranging from BaP at 100 µg to naphthalene at 300 x 10^3 µg per 100 g. Multiple water and sediment sampling, microbial-degradation studies, analysis of bioaccumulation by oysters, and analysis of adsorption to sediments with $[^{14}C]$PAH were carried out. The results demonstrated a rapid, marked decrease in PAHs from water (half-life, 3-4 d) and a variable recovery, depending on the PAH, in the sediment. For the low-molecular-weight PAH naphthalene, this recovery was only 7% after 1 wk; for BaP, it was 39%. Oysters rapidly took up all PAHs, but released naphthalene to such an extent that it was not detectable in the organisms after 23 d. In contrast, benz[a]anthracene and BaP were released much more slowly, with estimated half-lives (assuming exponential discharge) of 9 and 18 d, respectively. Thus, the higher-weight PAHs persisted much longer in the organisms than the lower-weight PAHs. Other degradation studies involving mussels collected from oil-contaminated waters also have shown the persistence of the higher-molecular-weight PAHs.[51,53] Evaporative loss of lower-weight PAHs, such as naphthalene, in the upper waters was expected, whereas this would be limited for higher-weight PAHs. Microbial degradation of naphthalene and anthracene was measurably increased in oil-contaminated water, compared with control water (4 h vs. 48 h, respectively, for appreciable degradation)--a finding consistent with those of other studies showing higher numbers of oil-degrading microorganisms in polluted than in control or unpolluted waters.[6,186] Photochemical degradation of PAHs was inferred; for BaP, this was considered to account for an amount that could approximate about 50% of the compound, inasmuch as no microbial degradation of the compound was demonstrated and 40% was recovered in the bottom sediment. The study permitted several conclusions that probably have general relevance. The half-lives of PAHs in marine waters are short (a few days); for lower-weight PAHs, microbial degradation and evaporative loss may be primary removal processes; for higher-weight PAHs, such as BaP, sedimentation and photodegradation are the most important removal means; and, by inference, for higher-weight PAHs after sedimentation, biologic degradation and interaction between plant and animal life in the sediment are important factors in removal.

These processes (biologic degradation and interactions) have been extensively studied with a wide variety of aquatic species. It is clear that, as with terrestrial fauna, the capacity of marine animal species to effect the metabolic transformation of PAHs can be considered to be universally distributed. Reviews of the results and other aspects of such studies have been published elsewhere,[3,4,37,38,129,181] and only representative reports are summarized here. PAHs in the marine environment can be metabolized by aquatic bacteria and fungi;[129] for some species of bacteria, a monocyclic aromatic hydrocarbon, such as benzene, can serve as a sole carbon source. PAHs, such as BaP and benz[a]anthracene, can also be oxidatively metabolized to hydroxylated derivatives comparable with those produced in the livers of vertebrates. PAHs can be degraded to CO_2 to a considerable degree (13-68%[129]) by aquatic microorganisms. PAH metabolism by fungi also occurs; these organisms contain cytochrome P-450 and can carry out the initial oxidative metabolism of PAHs in a manner resembling that catalyzed in vertebrate liver. Marine fungi isolated from oil-polluted water or oil slicks have a substantial ability to assimilate petroleum hydrocarbons, and this hydrocarbon-degrading capacity can permit use of a PAH as a growth substrate.[1]

Fish and crustaceans (and some worms) can oxidize PAHs--as measured by AHH activity--and cytochrome P-450 has been identified in a number of these species. Most oxidative metabolism in these aquatic animals is in the liver, as it is in mammals. Induction of cytochrome P-450 (not always correlated with an associated P-450-dependent increase in chemical oxidation) in fish has been produced by benz[a]anthracene, chrysene, BaP, and other organic substances[33,61,129,140,169] to which fish may be exposed in their natural environments or under experimental conditions. The products of the oxidative metabolism of PAHs in fish resemble those produced in mammalian liver and include diols, epoxides, phenols, quinones, and all principal types of conjugates formed from PAH metabolites in mammalian liver.

Seasonal changes in P-450-dependent oxidation have been reported in fish,[49] and alterations in this enzymatic activity have been related to ambient temperature, food status, and exposure to inducing chemicals in their natural habitat.[48,49] Apart from carrying out biotransformation, the capacity of marine species to accumulate and discharge PAHs from the surrounding waters is important in relation to the pattern of distribution of these compounds in the marine environment and to the use of marine species as food, in view of their contribution to the exposure of humans to PAHs via the gastrointestinal tract.

Marine animals readily accumulate PAHs from the surrounding waters and can discharge both the untransformed PAHs and their metabolic products into the aqueous environment. The rates of release of accumulated PAHs may vary substantially from species to species (and compound to compound), and half-lives can range from hours to many days. The substantial concentration gradients of PAHs that may occur between an organism and its aqueous environment can have importance for man in relation to marine species that are eaten by man or by edible species.[44,102,193] Whether these concentration gradients involve an active uptake mechanism is not known; but they do not depend solely on solubility, inasmuch as polar metabolites of a PAH can be retained longer than the more lipophilic parent compound.[13] This may be due to the electrophilic nature of these metabolites and their consequent binding to tissue macromolecules.[129]

Oysters have been shown to concentrate hydrocarbons from diesel-oil-contaminated waters to concentrations over 300 μg of total hydrocarbons per gram of wet weight over a 7-wk period.[170,171] These hydrocarbons were rich in aromatics, compared with the contaminating oil. In clean seawater, the hydrocarbon concentrations decreased dramatically (by 90% in 4 wk). Other marine species show the same biologic characteristics, although uptake and release of accumulated hydrocarbons vary. The concentration factor (i.e., tissue vs. water concentration) may reach 1,000-fold[23] in marine animals that cannot escape a contaminated environment. The potential importance for humans of this capacity for bioaccumulation in edible marine species is evident. PAHs can, as expected, accumulate rapidly in fish from contaminated sediments, as McCain _et al_.[118] have shown, although this process is less efficient than uptake from water.

The biologic impact of contaminating PAHs on marine species has been thoroughly reviewed recently[37,38,46,93,129] and is not summarized here. Toxic effects of these and related pollutants have been described across the spectrum of marine life, from bacteria and fungi to plants and animals; and they range from the "tainting" of commercial species[37,38] to the development of cancer and cancer-like growths in aquatic animals.[46,93]

PAHs in Food

The exposure of humans to PAHs from dietary constituents greatly exceeds that from any other sources except specific hazardous occupational settings. PAHs are ubiquitous contaminants of foods and--depending on the extent of atmospheric and soil pollution in crop areas and on methods of processing, preservation, and preparation--can become highly concentrated in selected foodstuffs. At least 100 types of PAHs have been identified in foods.[201] Some of these have been shown to have well-defined carcinogenic properties in experimental animals. Epidemiologic studies have suggested an association between the consumption of high-PAH foods and gastrointestinal malignancies in selected populations,[106,165,177] but it is difficult to extend this association to the general population or to define the biologic risk of PAHs in foods in more direct terms. Nevertheless, the quantitative dimensions of PAH exposure via the diet and the established carcinogenic potential of some of the compounds frequently identified in foods suggest that the health risks from this source of exposure, although still incompletely defined, may be important for various groups.

Edible marine species may contain variable amounts of PAHs derived principally from polluted terrestrial runoff waters, from marine sediments, and from petroleum-contaminated aquatic environments. As noted above, such environments are largely in-shore (e.g., estuaries and river basins), with pollution diminishing rapidly in the open seas. Bioaccumulation of PAHs in the marine food chain may be substantial in some fauna, and, of course, national predilections for such modes of seafood preparation as smoking[14,64,67,114,176] can increase to high values the content of PAHs in such foods. The potential for biomagnification of PAHs in aquatic food chains is clear, but the extent to which this process results in contamination of seafood ingested by humans is not known (the subject has been reviewed by Neff[129]). For some crustaceans and fish, PAH uptake through the food chain can be more efficient than uptake from the surrounding waters,[44,102,193] and storage of such compounds in these species can be substantial. PAHs thus stored may or may not undergo extensive biotransformation. The processes of storage, uptake, metabolism, accumulation, and excretion have generally large interspecies variation; but crustaceans appear relatively efficient in their uptake of PAHs from food and other sources.[129] Table 6-17 shows an analysis of PAHs in oysters collected in a moderately polluted harbor area by Cahnmann and Kuratsune.[30] The comparative BaP and benzanthracene contents of a variety of foodstuffs are shown in Table 6-18. (Also, see Table 6-21 for similar information on benzo[e]pyrene, chrysene, and dibenz[ah]anthracene.) The extent of and striking variation in PAH contamination of marine species are evident in the data (Table 6-19) of Mix and Schaffer,[126] who examined BaP concentrations in mussels (Mytilus edulis) in Yaquina Bay, Ore., at multiple sites over a 2-yr period. The variations have a time component, geographic determinants, seasonal and environmental elements, and unknown biologic influences that make generalizations from such data extremely difficult and perhaps impossible. The BaP concentrations in mussels reported by this study exemplify, however, the extent to which marine species have the potential for representing a considerable exposure source of PAHs in the human diet.

A variety of foodstuffs of terrestrial origin have been analyzed for PAH contamination, and many PAHs have been identified. They include the polycyclic compounds listed in Table 6-20, some of which have known carcinogenicity.[126] The known carcinogens 7,12-DMBA, cholanthrene, and dibenzo[ai]pyrene have also been identified in curing smoke. The relative concentrations of five carcinogenic PAHs in a sampling of foodstuffs are shown in Table 6-21.[201] It is clear from these data that amounts of some of these foodstuffs that are well within the amounts ingestible within a 1-d period constitute a PAH exposure via the gastrointestinal tract that can greatly exceed the pulmonary exposure of a very heavy smoker to PAHs.

Large amounts of PAHs can be found in soils and can enter food crops from this source. Table 6-22[25] shows results of a sampling of soils in the Northeast for BaP. The concentration of PAHs in soil can vary over an enormous range; for the prototype compound BaP, Baum[19] has summarized World Health Organization data showing a range (in micrograms per kilogram of soil) extending from around 100 (nonindustrial sites) through 1,000 (towns and vicinity) and 2,000 (soil near traffic) to 200,000 (soil near an oil refinery) or even over 600,000 (soil directly contaminated by coal-tar pitch). The higher figures reflect particle deposition, local atmospheric fallout, and direct waste discharge; the origin of the PAHs in forest samples (whose soil concentrations range up to 1,300 μg/kg[19]) is not certain, but must include a large contribution from natural combustion.

PAHs in food crops are probably derived in part from polluted soils, although the relative contribution of this source, compared with irrigation water or atmospheric pollution, is not established. PAHs in soils can be translocated to plants, probably through root adsorption, but the extent to which this occurs does not seem to correlate with the PAH content of soil.[89,159,184] Uptake of PAHs may also vary with plant species. The aboveground parts of edible plants can, of course, also concentrate PAHs through surface absorption from deposited dusts containing these compounds. Through this process, the aboveground parts of food crops can accumulate a gradient of PAH contamination exceeding that in root parts by a factor as high as 10,[89] and the bulk of this contamination in such edible crops as leafy vegetables (e.g., lettuce, spinach, and kale) and tomatoes cannot readily be removed by washing.[184] PAH contamination of irrigation wastes also contributes to an unknown extent to the contamination of edible plants. In the processing of foods, packing materials and additives are other sources of potential PAH contamination.

By far the largest sources of PAH contamination of foods are curing and preserving processes and cooking, especially of meats. Apart from shellfish, the "intrinsic" content of PAHs in most foods is low; for example, uncooked pork and beef may contain only 0.1 μg/kg. This concentration can increase substantially as a result of any cooking process (see Table 6-21) and especially as a result of smoking, curing, or broiling under a direct flame in which food drippings can be pyrolyzed.

PAH contamination of foods associated with smoke-curing results in part from the resinous condensates of liquid smoke flavors and from food combustion products.[64,106,150,177,191] The type of smoke generation and other characteristics of the smoking process can influence the amounts and types of PAHs produced--e.g., the temperature of combustion, the air supply, the length of smoke ducts, and the density and temperature of the smoke-cure.[177] Domestic smoking clearly produces more PAH than the commercial process,[14] probably because the procedure is less controlled and, as a result, entails heavier and more prolonged smoke exposure.[176] A general survey by the Food and Drug Administration and the U.S. Department of Agriculture of PAH content of smoked foods prepared commercially was reported by Malonoski et al.[112]

The broiling of meats over an open flame in which fat drippings can be pyrolyzed probably contributes more to diet-derived PAH exposure than any other method of food processing or preparation. Potent mutagens can be produced from amino acids and proteins in foods by high-temperature cooking.[36,116,127,167,173,196] This mode of cooking also increases the carcinogenic PAHs in meats to very high concentrations.[106]

The concentrations of 15 PAHs found in the outer surfaces of charcoal-broiled steaks by Lijinsky and Shubik[106] are recorded in Table 6-23. These concentrations are not unusually high for broiled or smoked meat (as the data in Table 6-21 indicate), nor for dietary constituents that are known to have a high PAH content, such as yeast, oils, some leafy vegetables and fruits, roasted coffee, and teas.[24,64,65,201] PAHs formed by pyrolysis can be derived (at least with pure substrates) from carbohydrates, fatty acids, and amino acids, and the extent of their production depends on temperature.[206] The data of Masuda et al.[115] (Table 6-24) show the amounts of 19 PAHs formed from combustion of six potential substrates at 500 or 700°C. Combustion took place in a nitrogen atmosphere; at 300°C, no PAHs were formed from any of the starting materials, but at the highest temperature studied, large amounts were produced from each. Clearly, substantial quantities of PAHs can be formed from these substrates under the pyrolytic conditions used, and, although ordinary pyrolysis takes place in air, the substrates tested are common constituents of foods and common broiling temperatures are within the range of those used in this study.

The conditions of broiling heavily influence the amounts of PAHs produced. Fatty meat produces more PAH after broiling than lean meat, and it has been suggested[106] that pyrolysis of fats dripping onto red-hot coals is the most likely source of PAHs. PAH production in broiled meat clearly depends, in addition, on the closeness of the meat to the heat source, on whether meat drippings reach the heat source (i.e., heating from the top, rather than the bottom), and on whether cooking is quick at high temperatures or slow at low temperatures.[104-107] Toxins other than PAHs are also produced by high-temperature cooking; these include the mutagenic-carcinogenic amino acid pyrolysis products described by Japanese and American workers and the N-nitroso compounds formed in cured-meat products, especially bacon and ham.[64] It should be noted that these non-PAH substances can be produced at temperatures distinctly lower than those used in conventional broiling and that a large fraction of them may be volatile; thus, redeposition of these airborne substances and their inhalation during cooking are additional toxin exposures that can be related to the diet.[146]

An approximate "balance sheet" of the estimated PAH exposure of humans from air, water, and food is shown in Table 6-25.[154] Despite a degree of inexactness in these figures--especially for foods--they provide a reasonable perspective of the sources of PAHs that might have an impact on man. It should be evident from these estimates that food constitutes the predominant source of PAHs for humans; even if the contribution from smoking were included, the diet would still be the dominant source.

The health impact of the PAHs in the human diet is not known, although, as noted above, an association between the intake of these substances in smoked foods and the occurrence of gastrointestinal malignancies in select populations has been inferred. The remarkably large amounts of PAHs that are ingested, compared with those to which the pulmonary system is exposed (even in heavy smokers), makes it clear that there must be tissue-specific factors related to the disposition of or metabolic responses to PAHs that protect the gut from the deleterious impact that might be anticipated from such exposure. The possibility of detrimental effects of diet-derived PAHs on the gastrointestinal system will not be so amenable to quantitation as has been the case with respect to smoking and the development of lung pathology.

An approach to defining the human metabolic impact of diet constituents in general and of charcoal-broiled meats in particular has been taken in the clinical-nutrition studies recently summarized by Anderson _et al_.[5] Several dietary factors were shown to influence potently the oxidative metabolism of various drugs used as model substrates for cytochrome P-450- and cytochrome P-448-mediated chemical transformations. It has been shown that isocaloric substitution of dietary protein for carbohydrate substantially shortens the plasma half-times of such drugs as antipyrine and theophylline; i.e., a protein-enriched diet increases the oxidative metabolism of these compounds. Opposite changes were observed during periods of high-carbohydrate feeding. Substitution of protein for fat in the diet (a nonisocaloric change) also stimulates the oxidative metabolism of these drug substrates; however, neither high-unsaturated-fat nor high-saturated-fat diets produce alterations in drug oxidation distinct from those produced by high-carbohydrate diets alone. Thus, with respect to influences on microsomal mixed-function oxidases, carbohydrate and fat in the diet appear to be interchangeable.

Feeding rats charcoal-broiled beef is known to increase intestinal metabolism of phenacetin.[137] Increased oxidative metabolism of this drug, as well as of antipyrine and theophylline, was also observed in the test subjects after short-term feeding (2 portions/d for 4 d) of normal portions of charcoal-broiled beef at mealtimes.[39,84,136] The effect of broiling (in control diets, the beef was protected from the cooking fire with aluminum foil) was striking; during the test-diet period, there was a pronounced decrease in the mean plasma concentration of phenacetin and a comparable decrease in the area under the curve for plasma phenacetin concentration plotted against time. The ratios of the mean concentrations of metabolite and unchanged phenacetin at each point studied increased markedly during the charcoal-broiled-beef test period, compared with control periods. The findings suggested that charcoal-broiled beef greatly stimulated the metabolism of this model drug substrate in the gastrointestinal tract or during its first pass through the liver. Smaller, but still substantial, increases in antipyrine and theophylline metabolism during the ingestion of the charcoal-broiled-beef test diet were also observed.

These systematic and pronounced effects of specific dietary manipulations on the metabolism of model drug substrates by the cytochrome P-450-dependent mixed-function oxidase system provide a valuable means for defining the metabolic responses of both normal and ill subjects to the ingestion of various foodstuffs or foods prepared in various ways. The physiologic import of such clinical studies can be greatly extended by the judicious selection of suitable chemical substrates for the metabolic systems under investigation. The extent to which individuality in man characterizes specific chemical biotransformations can also be explored by these metabolic techniques. Finally, it may be possible through such clinical studies--in which each subject serves as his own control--to identify patterns of biologic responses to specific foods or food components that might otherwise be obscured by the genetic and environmental diversity of large population groups.

TABLE 6-1

PAHs in Human Liver[a]

PAH	Concentration (wet basis), ppt 1 (F,54)	2 (F,17)	3 (F,65)	4 (M,65)	5 (M,51)	6 (M,41)
Anthracene	200	240	170	180	140	110
Pyrene	450	460	340	470	310	270
Benz[a]anthracene	ND	ND	ND	ND	ND	ND
Benzo[e]pyrene	ND	ND	ND	ND	ND	ND
Benzo[b]fluoranthene	88	81	87	68	53	33
Benzo[k]fluoranthene	15	23	10	17	8	6
Benzo[a]pyrene	13	21	19	22	10	11
Benzo[ghi]perylene	59	48	36	45	21	17
Dibenz[ah]anthracene	ND	ND	ND	ND	ND	ND

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[a] Reprinted with permission from Obana _et al_.[130] Numbers in parentheses are sex and age of subject. ND = not detectable.

TABLE 6-2

PAHs in Human Fat[a]

PAH	Concentration (wet basis), ppt 1 (F,54)	2 (F,27)	3 (F,66)	4 (M,65)	5 (M,51)	6 (M,41)	7 (F,35)	8 (M,52)	9 (M,35)	10 (M,66)
Anthracene	575	440	260	190	420	140	ND	25	140	390
Pyrene	780	920	890	650	1,500	590	49	2,000	1,300	2,700
Benz[a]anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Benzo[e]pyrene	71	110	64	57	140	83	49	30	41	150
Benzo[b]fluoranthene	260	190	240	77	250	120	56	95	110	160
Benzo[k]fluoranthene	28	40	35	17	43	27	ND	11	11	42
Benzo[a]pyrene	31	25	24	18	59	18	ND	12	16	19
Benzo[ghi]perylene	110	62	61	54	69	42	13	23	19	32
Dibenz[ah]anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

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[a] Reprinted with permission from Obana *et al*.[130] Numbers in parentheses are sex and age of subject. ND = not detected.

TABLE 6-3

Rates of Formation of Benzo[a]pyrene Metabolites by Hamster, Rat, and Human Microsomes[a]

Species and Organ	Animal Pretreatment	Total Metabolic Rate	Distribution of Metabolites,[c] % Dihydrodiols 1	 2	 3	 Quinones	 Phenols 1	 2
Rat liver (n = 3)	None	3,350.0	7.8	2.1	3.9	26.8	7.8	51.8
	PB	5,420.0	9.2	9.4	3.9	27.6	2.8	47.1
	3-MC	8,010.0	11.6	5.5	8.0	20.6	13.1	41.2
Hamster liver (n = 3)	None	4,380.0	2.5	15.5	2.7	29.7	5.0	44.6
	3-MC	3,320.0	3.0	20.5	2.7	33.1	4.2	36.5
Human liver (n = 9)	None	516.0	5.8	7.1	5.5	54.7	6.9	20.0
		340.0	2.4	1.5	1.9	6.0	3.1	4.6
Rat lung (n = 3)	None	23.2	4.7	7.8	6.5	30.6	15.9	34.5
	PB	28.9	3.8	6.2	6.6	29.4	16.6	37.4
	3-MC	112.8	15.2	8.0	9.7	14.5	14.7	37.9
Hamster lung (n = 3)	None	21.9	4.1	6.9	8.7	43.8	9.6	26.9
	3-MC	59.0	9.0	5.9	9.3	36.8	5.1	33.9
Human lung (n = 19)	None	11.5	9.3	6.4	12.5	33.9	11.0	26.9
		4.8	5.1	3.6	7.0	13.0	7.2	9.7
Human kidney (n = 10)	None	16.5	4.7	5.5	5.0	57.3	8.0	17.9
		10.2	3.3	3.2	2.5	11.6	1.4	6.1

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[a] Reprinted with permission from Prough *et al.*[143]

[b] Means, expressed as picomoles of product formed per minute per milligram of microsomal protein.

[c] Grouped into three classes of dihydrodiols and two classes of phenols, because the radioactive peaks cochromatographed with the authentic standards of the dihydrodiols (dihydrodiol 1, 9,10-dihydrodiol; dihydrodiol 2, 4,5-dihydrodiol-BaP; and dihydrodiol 3, 7,8-dihydrodiol) and phenols (phenol 1, 9-hydroxy-BaP, and phenol 2, 3-hydroxy-BaP).

TABLE 6-4

Metabolism of Benz[a]anthracene, 7,12-Dimethylbenz[a]anthracene, and Benzo[a]pyrene by Human Mammary Tissue[a]

Hydrocarbon	Patient	Protein, mg	Water-soluble Metabolites, nmol/mg protein	Ether-soluble Metabolites, nmol/mg protein	Protein-bound Metabolites, pmol/mg protein	Hydrocarbon-DNA Adducts, pmol/mg protein	Total Metabolism of Administered Hydrocarbon, %
Benz[a]anthracene	6	19.7	24.0	1.1	72	<0.1	30.8
	7	22.5	3.3	2.2	129	<0.1	8.0
	8	22.2	22.2	4.5	30	<0.1	27.9
7,12-Dimethylbenz[a]anthracene	1	64.3	4.6	1.7	260	0.5	26.6
	2	33.3	9.9	1.6	47	2.7	21.1
	3	20.5	20.3	1.7	371	1.8	28.7
Benzo[a]pyrene	3	16.0	12.0	1.0	28	0.4	13.1
	4	18.8	8.4	1.0	47	0.7	11.1
	5	78.4	1.6	0.1	90	0.7	8.9

---

[a] Reprinted with permission from Grover et al.[66] Metabolism and activation measured with epithelial-cell aggregates in culture prepared from nonneoplastic mammary tissue.

TABLE 6-5

Binding of [^{3}H]Benzo[a]pyrene to DNA in Human Endometrial Tissue Taken Throughout Menstrual Cycle and Before and After Menopause[a]

Hormonal Status	[^{3}H]B[a]P Binding to DNA, dpm/µg DNA Mean ± S.E.	No. Cases
Early and midproliferative	15.0 ± 3.69	11
Late proliferative and early secretory	24.5 ± 6.12	16
Midsecretory and late secretory	6.7 ± 2.12	10
Premenopausal	16.8 ± 2.70	37
Postmenopausal	4.7 ± 1.67	3

---

[a] Reprinted with permission from Dorman *et al*.[52]

TABLE 6-6

Effect of PAHs in Cigarette Smoke on Benzopyrene Hydroxylase Activity in Rat Placenta[a]

PAH	8-Hydroxybenzopyrene formed, ng/g-h
Control	218 ± 81
1,2-Benzanthracene	4,034 ± 519
1,2,5,6-Dibenzanthracene	3,577 ± 494
3,4-Benzopyrene	3,543 ± 114
Chrysene	3,267 ± 117
3,4-Benzofluorene	1,939 ± 98
Anthracene	1,377 ± 316
Pyrene	1,232 ± 306
Fluoranthene	1,123 ± 129
Perylene	805 ± 159
Phenanthrene	721 ± 155

---

[a] Reprinted with permission from Welch *et al.*[190]

[b] Rats pregnant for 18 d were given PAH orally at 40 mg/kg. Placenta was assayed for benzopyrene hydroxylase activity 21 h after the dose. Each value represents the mean ± S.E. from three rats.

TABLE 6-7

Variability in Induction of Benzopyrene Hydroxylase Activity in Human Placenta[a]

Subject	Hydroxybenzopyrene Formed by Placenta, ng/g-h
L.B.	240
G.A.	260
P.C.	547
C.G.	643
A.T.	1,269
J.K.	1,317
L.C.	1,860
C.J.	4,289
E.R.	4,390
D.B.	5,267
D.A.	15,181
H.J.	16,524
M.N.	17,100

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[a] Reprinted with permission from Conney *et al*.[42] All subjects in this study were Caucasian, and all smoked 15-20 cigarettes daily during pregnancy. Variability in benzopyrene hydroxylase activity was not related to medication taken during or before delivery.

TABLE 6-8

Specific Activities of Binding of Tritium-Labeled PAHs to Human Bronchial DNA[a]

PAH	No. Cases	Specific Activity	
		dpm/μg of DNA	pmol/mg of DNA
7,12-Dimethylbenzanthracene	3	170 ± 22	53 ± 7
Benzo[a]pyrene	4	224 ± 77	40 ± 14
3-Methylcholanthrene	2	38 ± 9	34 ± 8
Dibenz[ah]anthracene	3	15 ± 3	28 ± 6

---

[a] Reprinted with permission from Harris *et al.*[70] *Nature*, Vol. 252, pp. 68-69, copyright 1974 Macmillan Journals Limited.

[b] Mean ± S.E. Amount of DNA and dpm determined from peak DNA fraction of CsCl gradients.

TABLE 6-9

Disappearance of PAHs from Soot in Human Lungs[a]

Lung No.	Pt. Age, yr	Lung Weight, g (wet wt.)	Soot, mg	Ash, mg	Pyrene		Benzopyrene and Benzoperylene	
					Found, μg	Expected, μg	Found, μg	Expected, μg
2	90	950	720	480	0.9	6-27	--	34-172
3	71	1,335	360	200	1.9	3-13	--	17-86
4	70	1,570	660	770	1.9	6-24	--	31-158
5	74	1,570	360	170	3.6	3-13	--	17-86
6	71	710	390	1,070	1.3	3-14	--	18-93
7	81	1,260	190	150	2.3	2-7	--	9-45
8	62	1,120	540	510	4.4	5-20	--	25-130
9	70	910	830	310	5.1	7-31	Trace	38-200
11	65	1,420	5,010	3,460	1.3	45-185	--	233-1,200

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[a] Reprinted with permission from Falk _et al_.[56]

TABLE 6-10

PAHs in Human Lung Samples from Surgical Operations[a]

Sample No.	Tissue	Weight of Sample, g	Benzo[a]pyrene Total, ng	Benzo[a]pyrene ng/g	Fluoranthene Total, ng	Fluoranthene ng/g	Benzo[b]fluoranthene Total, ng	Benzo[b]fluoranthene ng/g	Perylene Total, ng	Perylene ng/g
1	Bronchial carcinoma	20.0	300,000	15,000	<8.8	--	<8.0	--	<2.3	--
2	Bronchial carcinoma	25.0	3,300	130	<8.8	--	<8.0	--	<2.3	--
3	Bronchial carcinoma	35.0	34,000	900	<8.8	--	<8.0	--	<2.3	--
4	Bronchial carcinoma	20.0	1,640	80	<8.8	--	<8.0	--	<2.3	--
5	Bronchial carcinoma	23.0	5,760	250	400	17	<8.0	--	<2.3	--
6	Bronchial carcinoma	8.0	690	80	<8.8	--	<8.0	--	<2.3	--
7	Bronchial carcinoma	18.9	80.0	4.0	63.6	3.4	<8.0	--	1,840	97.2
8	Bronchial carcinoma	26.5	220.0	8.3	<8.8	--	<8.0	--	2,160	81.4
9	Bronchial carcinoma	27.0	80.0	3.0	<8.8	--	<8.0	--	<2.3	--
10	Bronchial carcinoma	43.4	93.3	2.2	<8.8	--	<8.0	--	<2.3	--
11	Bronchial carcinoma	66.4	570.0	8.7	<8.8	--	<8.0	--	7,670	12.0
12	Bronchial carcinoma	39.8	97.7	2.4	<8.8	--	<8.0	--	480	12.0
13	Bronchial carcinoma	31.2	91.7	2.9	<8.8	--	<8.0	--	290	9.2
14	Bronchial carcinoma	39.7	<2.2	--	<8.8	--	<8.0	--	<2.3	--
15	Bronchial carcinoma	67.8	<2.2	--	<8.8	--	<8.0	--	<2.3	--
16	Bronchial carcinoma	41.0	290.0	7.1	<8.8	--	410	10	<2.3	--
17	Bronchial carcinoma	148.1	640.0	4.3	<8.8	--	<8.0	--	<2.3	--
18	Bronchial carcinoma	112.4	28.7	0.3	<8.8	--	<8.0	--	<2.3	--
19	Bronchial carcinoma	116.7	190.0	1.6	850	7.3	1,080	9.3	<2.3	--
20	Bronchial carcinoma	114.0	100.0	0.9	<8.8	--	910	8.0	<2.3	--
21	Bronchial carcinoma	87.4	96.6	1.1	<8.8	--	710	8.2	<2.3	--
22	Adjoining tissue	53.0	<2.2	--	<8.8	--	<8.0	--	<2.3	--
23	Adjoining tissue	97.0	<2.2	--	<8.8	--	<8.0	--	<2.3	--
24	Adjoining tissue	26.0	<2.2	--	<8.8	--	<8.0	--	<2.3	--
25	Adjoining tissue	62.0	<2.2	--	<8.8	--	<8.0	--	<2.3	--
26	Adjoining tissue	24.0	<2.2	--	<8.8	--	<8.0	--	<2.3	--
27	Adjoining tissue	42.0	<2.2	--	<8.8	--	<8.0	--	<2.3	--
28	Adjoining tissue	32.0	<2.2	--	<8.8	--	<8.0	--	<2.3	--
29	Adjoining tissue	51.0	<2.2	--	<8.8	--	<8.0	--	<2.3	--
30	Adjoining tissue	54.0	<2.2	--	<8.8	--	<8.0	--	<2.3	--

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[a] Reprinted with permission from Tomingas *et al*.[178]

TABLE 6-11

Environmental and Occupational Benzo[a]pyrene and Total Suspended Particulate Concentrations[a]

Location	Benzo[a]pyrene, $\mu g/1,000\ m^3$	Total Suspended Particles,[b] $\mu g/m^3$
28 rural sites, U.S.	0.01-1.9, annual avgs.	--
94 urban sites, U.S., 1958-1959	0.11-61.0, annual avgs.	--
All rural sites, U.S.	--	28, arith. avg., biweekly samples
291 urban sites, U.S., 1960-1965	--	105, arith. avg., biweekly samples (1,254 maximum)
Merton bus garage, June 1956	26.5 (inside), 7-h mean 14.5 (outside), 7-h mean	210-1,440 (inside) 140-240 (outside)
Blackwall Tunnel, London, summer, 1958	350, daytime mean	930-2,350
Sumner Tunnel, Boston, summer, 1961	690, 24-h mean	588 (outlet) 104 (inlet)
Diesel locomotive cab	--	380 (100-800)
Diesel roundhouse	--	1,990 (70-8,700)
Coal and pitch coking plants	300-35,000, short-term sample	--
Gasworks retort houses	3,000, long sample means	--
Roof tarring, outdoor	90-14,000, short-term samples	--

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[a] Reprinted with permission from Schenker.[155]

[b] For 24-h samples collected by high-volume filters.

TABLE 6-12

Epidemiologic Studies of Cancer in Occupations Exposed to Diesel Exhaust[a]

Population Studied	Findings	Comment
Male London transportation workers, 45-65 yr old, 1950-1955	96 cases lung cancer; no excess lung cancer attributed to diesel-exhaust exposure	Inadequate duration of exposure and latent period at time of study; diesel-exhaust exposure estimated
Baltimore and Ohio Railroad workers, 19[illegible]-1958	154 lung-cancer deaths; SMR slightly lower than national rates; no differences in rates for exposed and nonexposed workers	Inadequate duration of diesel-exhaust exposure and latent period at time of study; no consideration of transfers, retirement, or duration of exposure
"Two large railroad companies," 1939-1950	133 cases lung cancer; about 3:1 ratio observed:expected in operating workers, compared with nonoperating workers	Inadequate duration of diesel-exhaust exposure at time of study; rates not calculated
Finnish railroad workers, 30-52 yr old, 1955-1973	47 tumors in engineers--significantly greater number than in trainmen or railroad clerks	Small number of cases; not analyzed by tumor type
Massachusetts tumor registry, 1965-1972	91 cases oat-cell cancer; excess in transportation-equipment operatives	Not specific for diesel-exhaust exposure
Central state teamsters, May-July 1976	34 respiratory-tract cancer deaths; increased SMR all age groups; significant for age 50-59	Small number of cases; short period of observation

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[a] Reprinted with permission from Schenker et al.[155]

TABLE 6-13

Benzo[a]pyrene Content of Shale Materials,[a] μg/kg (ppb)

Raw Shale			Spent Shale			Retort Oil				
							Parent		Methyl-Substituted	
Shale	1st	2nd	Shale	1st	2nd	Oil	1st	2nd	1st	2nd
RS-101	2.5	1.9	SS-201	ND < 0.4	ND < 0.2	RO-1	1,900	1,700	5,000	3,300
RS-102	2.0	1.6	SS-202	19.8	19.6	RO-2	1,800	1,800	21,000	22,000
RS-103	1.7	1.6	SS-203	23.8	23.3	RO-3	2,300	2,300	11,000	12,000

---

[a] Reprinted with permission from Weaver and Gibson.[187]

TABLE 6-14

PAHs in Shale Retort Oils,[a] ppb

Component	Parent	Methyl-Substituted
Pyrene	17,500	50,500
Fluoranthene	5,650	8,050
Benz[a]anthracene	1,200	12,000
Chrysene	2,850	23,500
Triphenylene	540	5,700
Benzo[a]pyrene	4,250	8,350
Benzo[e]pyrene	1,950	2,650
Perylene	325	1,015
Anthanthrene	275	455
Benzo[ghi]perylene	1,900	8,650
Coronene	--	--

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[a] Reprinted with permission from Weaver and Gibson.[187]

TABLE 6-15

PAHs in Drinking Water[a]

Source	Concentration, ng/L BaP	 Carcinogenic PAH	 Total PAH
Mixed tap water at Mainz, Germany	--	--	7.0
Water at:[b]			
Syracuse, N.Y.	0.3	0.3	1.1
Buffalo, N.Y.	0.2	0.2	0.9
New York, N.Y.	0.5	3.9	6.4
Lake George, N.Y.	0.3	1.5	4.2
Endicott, N.Y.	0.2	1.1	8.3
Hammondsport, N.Y	0.3	1.5	3.5
Pittsburgh, Pa.	0.4	1.9	2.8
Philadelphia, Pa.	0.3	2.0	14.9
Huntington, W.Va.	0.5	2.0	7.1
Wheeling, W.Va.	2.1	11.3	138.5
New Orleans, La.	1.6	1.6	2.2
Appleton, Wis.	0.4	2.4	6.1
Champaign, Ill.	ND[c]	1.2	2.8
Fairborn, Ohio	0.1	0.8	2.9
Elkhart, Ind.	ND[c]	0.3	0.3

[a] Reprinted with permission from Santodonato *et al.*[154]

[b] Only the six W.H.O.-recommended PAHs were analyzed, with the exception that BjF replaced BbF.

[c] ND = not detected.

TABLE 6-16

PAHs in Tapwater[a]

PAH	Concentration, ppt
Naphthalene	2.9
2-Methylnaphthalene	1.4
1-Methylnaphthalene	1.1
Biphenyl	0.32
Acenaphthene	0.82
Dibenzofuran	0.62
Fluorene	0.72
Dibenzothiophene	0.21
Phenanthrene	3.1
Anthracene	0.35
2-Methylanthracene	0.06
4,5-Methylenephenanthrene	0.30
1-Methylphenanthrene	0.37
Fluoranthene	2.6
Pyrene	1.1
Benzo[a]fluorene	0.05
Benzo[b]fluorene	0.05
4-Methylpyrene	0.05
Methylpyrene	0.08
1-Methylpyrene	0.05
Benz[a]anthracene	0.49
Benzo[b]fluoranthene	0.21
Benzo[jk]fluoranthenes	0.07
Benzo[e]pyrene	0.20
Benzo[a]pyrene	0.05

---

[a] Data from Olufsen.[132]

TABLE 6-17

PAHs in Extracts from Shucked Oysters[a]

Compound	Approximate Concentration, µg/5 kg of oysters
Benzo[ghi]perylene	5-25
Benzo[a]pyrene	10-30
Benz[a]anthracene	50
Benzo[k]fluoranthene	40-60
Benzo[e]pyrene	100
Chrysene	100-200
Pyrene	500-800
Fluoranthene	3,000-5,000

---

[a] Reprinted with permission from Cahnmann and Kuratsune;[30] copyright 1957 American Chemical Society.

TABLE 6-18

PAHs in Foodstuffs[a]

Foodstuffs	Concentration,[b] μg/kg (wet wt.) Benzo[a]pyrene	Benzanthracene
Cooked meats, sausage	0.17-0.63	0.2-1.1
Cooked bacon	1.6-4.2	--
Charcoal-broiled meats	2.6-11.2 (50.4 recorded)	1.4-31
Smoked ham, sausage	0.02-14.6	0.4-9.6
Heavily smoked ham	Up to 23 (107 recorded, Iceland)	Up to 12
Cooked fish	0.9	Up to 2.9
Smoked fish	0.3-60 (up to 37 in Japan)	0.02-2.8 (up to 189 in Japan)
Cereal grains	0.2-4.1	0.4-6.8
Flour and bread	0.1-4.1	0.4-6.8
Bakers' dry yeast (yeasts grown on mineral oils are lower)	1.8-40.4	2.9-93.5
Soybean	3.1	--
Refined vegetable oils, fats	0.4-36	0.8-1.1
Margarine, mayonnaise	0.2-6.8	1.4-29.5
Salad	2.8-12.8	4.6-15.4
Tomatoes	0.2	0.3
Spinach	7.4	16.1
Kale (only 10% removed by washing)	12.6-48.1	43.6-230
Apples	0.1-0.5	--
Fruits (not apples)	2-8	--
Dried prunes	0.2-1.5	--
Roasted coffee and solubles	0.1-4	0.5-14.2
Malt coffee	Up to 15	Up to 43
Tea	3.7-21.3	--
Whiskey	0.04 μg/L	0.04-0.08 μg/L
Beer	ND	ND
Roasted peanuts	--	Up to 0.95
Milk	ND	--

---

[a] Reprinted with permission from U.N. Food and Agriculture Organization.[180]
[b] ND = not detected.

TABLE 6-19

Benzo[a]pyrene in *M. edulis* from Yaquina Bay, Oregon[a]

Date	Benzo[a]pyrene Concentration, μg/kg at Site:[b]												
	Y1M	Y2M	Y3M	Y4M	Y5M	Y6M	Y7M	Y8M	Y10M	Y11M	Y12M	Y13M	Y14M
6/15/76	0.1	30	8.1	15	0.9	3.0	4.1	0.4	5.2	0.5	0.4	0.4	4.3[c]
7/22/76	4.7	67	4.5	6.7	4.4	2.3	2.4	0.8	10	0.6	0.7	0.3	0.5
9/24/76	0.7	34	16	6.9	1.2	1.9	14	0.9	6.3	NS	0.8	0.8	0.3
11/16/76	0.6	40	8.4	8.9	2.7	17	19	NS	3.6	0.4	0.4	0.9	0.6
12/16/76	8.4[c]	12	7.2	7.5	0.6	6.1	3.8	NS	2.8	NS	0.3	0.1	0.4
2/03/77	3.8	33	71[c]	170[c]	0.9	8.1	1.7	2.0	3.0	0.7	0.5	0.2	0.2
4/08/77	1.7	22	7.9	12	1.5	4.4	NS	NS	3.8	0.7	0.5	NS	0.4
6/29/77	6.3	15	3.5	5.4	2.1	50[c]	3.5	1.5	NS	2.0	0.4	0.0	0.4
8/29/77	1.2	5.1	2.8	2.2	5.6	4.4	5.8	NS	NS	0.4	0.0	2.6	0.3
10/13/77	0.8	5.0	5.3	1.9	1.2	3.2	4.2	0.5	4.2	0.1	0.2	0.4	0.34
12/08/77	1.2	15	NS	6.4	4.7	3.1	36	8.1[c]	9.4	NS	0.0	NS	NS
2/03/78	3.1	27	NS	13	7.7	32[c]	NS	2.3	10	3.0	1.2	NS	NS
4/28/78	0.7	20	NS	5.5	1.2	4.0	27	2.7	NS	NS	0.1	NS	NS
6/24/78	0.8	29	NS	17	NS	NS	NS	NS	NS	NS	NS	NS	NS
Average	2.0	26	6.5	8.5	2.7	7.5	11	1.4	6.0	1.0	0.4	0.7	0.4

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[a] Reprinted with permission from Mix and Schaffer.[126]

[b] NS = not sampled or not yet analyzed.

[c] Data not included in statistical analyses because of large variation (>4x) from the mean.

TABLE 6-20

PAHs in Foods[a]

1	Anthracene
2	Benzanthracene*
3	Methylbenzanthracene
4	Dibenz[aj]anthracene*
5	Dibenz[ah]anthracene*
6	Dibenz[ac]anthracene*
7	Dibenz[ai]anthracene*
8	Phenanthrene
9	3-Methylphenanthrene
10	2-Methylphenanthrene
11	9-Methylphenanthrene
12	2,6-Dimethylphenanthrene
13	Fluorene
14	Benzo[a]fluorene
15	Benzo[b]fluorene
16	Benzo[a]fluoranthene
17	Benzo[b]fluoranthene
18	Benzo[j]fluoranthene*
19	Benzo[k]fluoranthene
20	Benzo[ghi]fluoranthene
21	Pyrene
22	4-Methylpyrene
23	_o_-Phenylenepyrene*
24	Benzo[a]pyrene*
25	Benzo[e]pyrene*
26	Dibenzo[ah]pyrene*
27	Anthanthrene
28	Chrysene*
29	Perylene
30	Benzo[ghi]perylene
31	Acenaphthene
32	Acenaphthylene
33	2-Methylnaphthalene
34	Naphthalene
35	Acenaphthalene

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[a] Reprinted with permission from Mix and Schaffer.[126] Asterisk indicates known carcinogenicity.

TABLE 6-21

PAHs in Foodstuffs[a]

Foodstuff	Compound	Concentration, μg/kg
Broiled sausage	Benz[a]anthracene	0.2-1.1
Smoked sausage		0.4-9.9
Heavily smoked ham		12
Spinach		16
Crude coconut oil		98
Refined vegetable oil		1
Broiled sausage	Benzo[a]pyrene	0.17-0.63
Charcoal-broiled meat		2.6-11.2
Smoked fish		2.1
Spinach		7.4
Tomatoes		0.2
Crude coconut oil		43.7
Roasted coffee		0.1-4
Tea		3.9-21.3
Cereals		0.2-4.1
Smoked ham	Benzo[e]pyrene	5.2
Smoked fish		1.9
Spinach		6.9
Tomatoes		0.2
Crude coconut oil		32.7
Roasted coffee		0.3-7.2
Roasted peanuts		0.4
Broiled sausage	Chrysene	0.5-2.6
Heavily smoked ham		21.2
Spinach		28
Tomatoes		0.5
Cereals		0.8-14.5
Roasted coffee		0.6-19.1
Black tea		4.6-6.3
Spinach	Dibenz[ah]anthracene	0.3
Tomatoes		0.04
Cereals		0.1-0.6

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[a] Reprinted with permission from Zedeck.[201]

TABLE 6-22

Benzo[a]pyrene in Soils[a]

Benzo[a]pyrene Origin and Type of Soil	Concentration, μg/kg
Oak forest, West Falmouth, Mass.	40
Pine forest, West Falmouth, Mass.	40
Mixed forest, West Falmouth, Mass.	1,300
Mixed forest, eastern Conn.	240
Garden soil, West Falmouth, Mass.	90
Plowed field, eastern Conn.	900

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[a] Reprinted with permission from M. Blumer, Science 134: 474-475, 1961;[25] copyright 1961 by the American Association for the Advancement of Science.

TABLE 6-23

PAHs in Charcoal-Broiled Steaks[a]

PAH	Concentration, µg/kg of steak
Alkylbenzanthracene	2.4
Anthanthrene	2
Anthracene	4.5
Benz[a]anthracene	4.5
Benzo[b]chrysene	0.5
Benzo[ghi]perylene	4.5
Benzo[a]pyrene	8
Benzo[i]pyrene	6
Chrysene	1.4
Coronene	2.3
Dibenz[ah]anthracene	0.2
Fluoranthene	20
Perylene	2
Phenanthrene	11
Pyrene	18

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[a] Reprinted with permission from Lijinsky and Shubik.[106]

TABLE 6-24

PAHs Produced by Pyrolysis of Carbohydrates, Amino Acids, and Fatty Acids at Two Temperatures[a]

PAH	PAH Concentration, µg/50 g											
	Starch		D-Glucose		L-Leucine		L-Glutamic acid		Palmitic acid		Stearic acid	
	700 C	500 C	700 C	500 C	700 C	500 C	700 C	500 C	700 C	500 C	700 C	500 C
Naphthalene	5,140	--	16,000	--	6,500	--	1,800	--	223,000	--	19,000	--
Acenaphthylene	300	--	4,410	--	732	--	395	--	49,100	--	40,400	--
Fluorene	1,550	--	1,260	--	1,450	--	218	--	8,150	--	11,600	--
Anthracene	814	32	1,240	49	632	--	357	--	13,500	--	8,880	--
Phenanthrene	1,560	48	2,440	66	2,200	--	582	--	38,100	--	50,100	--
Pyrene	965	41	1,680	23	1,200	--	755	1.5	17,600	0.5	18,700	0.7
Fluoranthene	730	13	1,200	19	320	--	870	0.5	6,700	0.1	6,590	0.5
Benzo[a]fluorene	306	15	165	23	155	--	16	--	2,470	--	3,410	--
Benz[a]anthracene	315	22	520	25	270	--	119	--	5,710	--	8,410	--
Chrysene	159	6	210	8	48	--	32	--	2,710	--	4,550	--
Perylene	24	2	45	1	13	--	8	--	460	--	675	--
Benzo[a]pyrene	179	7	345	6	77	--	58	--	3,750	--	4,440	--
Benzo[e]pyrene	82	3	175	1	20	--	43	--	2,390	--	2,630	--
Benzo[b]fluoranthene	62	--	150	--	17	--	13	--	--	--	--	--
Benzo[j]fluoranthene	32	--	160	--	53	--	20	--	--	--	--	--
Benzo[k]fluoranthene	48	--	120	--	7	--	12	--	210	--	352	--
Benzo[ghi]perylene	134	3	180	2	40	--	35	--	1,550	--	2,740	--
Anthanthrene	49	2	150	--	20	--	18	--	850	--	935	--
Coronene	9	--	10	--	3	--	--	--	95	--	52	--

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[a] Reprinted with permission from Masuda et al.[115]

TABLE 6-25

Estimated Daily Human Exposure to PAH from Air, Water, and Food[a]

Source	Benzo[a]pyrene, μg	Total PAH, μg
Air	0.0095-0.0435	0.207
Water	0.0011	0.027
Food	0.16-1.6	1.6-16

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[a] Reprinted with permission from Santodonato *et al.*[154]

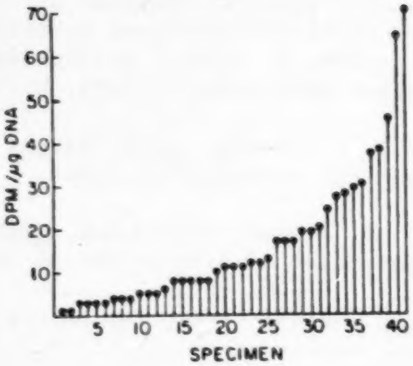

FIGURE 6-1. Spectrum of specific activities of binding of [^{3}H] to DNA in human endometrial tissue in vitro. Human endometrial tissue was incubated for 18 hr in organ culture in medium containing 1 M[^{3}H)BP. For each of the 41 specimens of endometrial tissue examined, specific activities of binding were determined in order to most clearly illustrate the range of binding of [^{3}H] to DNA in endometrial tissue from these patients. This histogram has been constructed with cases enumerated in increasing order of specific activity. Reprinted with permission from Dorman et al.[52]

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7

SOME FACTORS THAT AFFECT SUSCEPTIBILITY OF HUMANS TO POLYCYCLIC AROMATIC HYDROCARBONS

The interaction of chemical pollutants, including the PAHs, and mammalian cells can result in a variety of problems, including toxicity, mutagenesis, carcinogenesis, and teratogenesis. This interaction of chemicals with somatic cells probably results in such end points as cancer, and the interaction of chemicals with germ cells probably results in a variety of hereditary disorders. Many genetic disorders result in a predilection to the development of cancer. The cancer burden in the male population in the United States, although speculative, is distributed approximately as follows: 40% from tobacco-smoking, 10-20% from all diet-related causes, 5% from occupational exposures, 5% from single-gene inheritance, and 35% from other causes, which may include unknown genetic predisposition and environmental effects.[59] The birth-defects burden in the United States is distributed approximately as follows: 5-10% from known teratogens, such as viruses, chemicals, and radiation; 25% from genetic anomalies; and 60-65% from unknown mixtures of genetic predisposition and environmental effects.[59] Although monogenic disorders (including dominants), X-linked recessive disorders, and chromosomal abnormalities account for only about 5% of the human disease burden, the impact of heterozygous recessively inherited abnormalities similar to the monogenic disorders is very ill-identified, but could outweigh all other contributions.[115]

The heterozygous recessively inherited disorders may be the major reason why cancer incidences are not uniformly distributed.[95,97] In fact, of the millions of people exposed to such environmental chemicals as diethylstilbestrol, estrogen oral contraceptives, vinyl chloride, and cigarette smoke, only a very small proportion develop or express the cancer thought to be associated with these exposures. It is likely that genetic variability within the human population accounts in part for the distribution pattern. As depicted in Figure 7-1, cancer sensitivity can be viewed as a function of inborn susceptibility. Where this inborn or genetic susceptibility is low, cancer expression is low. Where this susceptibility is high (e.g., in single-gene defects), cancer expression is high. The major question is whether the combination of chemical exposure and genetic susceptibility can change significantly the numbers of persons who develop cancer.

PAHs are ubiquitous chemicals capable of producing a broad spectrum of biologic responses. Some can cause cancer in a variety of tissues, including lung, liver, kidney, colon, skin, and bladder. In humans, epidemiologic evidence has demonstrated that the incidences of cancers of stomach, nasal cavity and sinuses, lung, and to a lesser extent rectum, testis, skin (e.g., melanoma), brain, liver, pancreas, and hemopoietic tissue (i.e., leukemia) are correlated with areas containing high concentrations of industrial pollutants.[10,46,102,182,183] For many persons, the amount of these agents in the environment may be the rate-determining factor for cancer susceptibility. Thus, the primary need would be to identify and measure the amount of exposure to the environmental pollutants. The advent of a variety of in vitro and in vivo bioassays promises the development of methods for identifying chemicals that are potential carcinogens.

In animal-model systems, susceptibility to chemically induced cancers is usually dose-related. However, route, duration, and frequency of administration and such genetic factors as species, sex, and strain all tend to modify the relationship. In humans, mixtures containing PAHs can certainly cause cancer, but inadequacies in the information on age and trauma but especially on duration, frequency, and intensity of exposure and on the size and characteristics of the exposed population make quantitative estimation of dose-response relationships and the concept of thresholds difficult to interpret.

EFFECT OF GENETIC DIFFERENCES

The hypothetical stages in carcinogenesis are depicted in Figure 7-2. PAHs probably can show biologic effects at any of these stages. Thus, answers are needed to the following questions: Which stages can PAHs modify in humans? Are there naturally occurring variations in the expression of some of these steps in humans? Can a genetic basis be identified for the regulation of these naturally occurring differences? If so, can the differences result from the action of a single gene system? Can a relationship be shown between the expression in the gene locus and PAH-mediated effects?

PAH-induced effects in humans could depend on exposure, uptake, and distribution of the chemicals; their metabolic activation and inactivation; DNA-repair capacity; "promoters"; and the extent of immunocompetence. Each of these is discussed below.

UPTAKE AND DISTRIBUTION OF PAHs IN TISSUES

The distribution of PAHs in tissues or cells depends on the route of exposure. According to the results of Rees *et al.*,[152] the distribution of benzo[a]pyrene (BaP) in tissue other than at the site of absorption (i.e., intestine) depends on two phases: accumulation of the BaP on the tissue and passive diffusion through the tissue. These two phases underlie these authors' views about the apparent exponential nature of the accumulation of BaP as a function of dose. The exponential increase could be very important, but it must be pointed out that humans are rarely exposed to BaP at concentrations greater than 200 μM (i.e., 50 μg/ml) under "normal" circumstances. Concentrations of a variety of PAHs (e.g., pyrene, anthracene, and BaP) in human tissues average about 1,100 parts per trillion (ppt) in fat tissue and 380 ppt in liver.[119] BaP can vary from 0.3 to 15,000 parts per billion (ppb) in bronchial-carcinoma tissue.[184] Most of the subjects in the study of Tomingas et al.[184] were cigarette-smokers, but no obvious correlation between BaP concentration and extent of smoking was seen. The PAHs observed in addition to BaP included fluoranthene, benzo[b]fluoranthene, and perylene.

Reasons for differences in tissue distribution are not known, but, inasmuch as most of these chemicals are inducers and substrates for microsomal enzymes, tissue variation in cytosol and nuclear receptors could be important. In rodents, the induction of the microsomal monooxygenase system by some PAHs depends on the presence of particular cytosol receptor proteins.[56,142,143] These receptor proteins are not evenly distributed in all tissues, but are highest in thymus and lung, lower in liver and kidney, lower yet in testes, brain, and skeletal muscle, and not detectable in pancreas, adrenal, or prostate.[15] Most importantly, receptor proteins are found in high concentrations in strains of animals or cultured cells in which PAHs induce the enzyme aryl hydrocarbon hydroxylase (AHH) and are nondetectable in those in which AHH is nonresponsive.[56,142] This correlation also extends to humans, in whom the concentration of a BaP-binding plasma component is correlated with the capacity of lymphocytes to be induced for AHH activity in culture.[104] A cytoplasmic receptor for BaP, which did not cross-react with 7,12-dimethylbenz[a]anthracene, has also been reported for human cells in culture.[31] The presence of some of these receptors is under specific genetic control in animal models,[122,143], so uptake and distribution, at least in particular persons, could be under a form of genetic control.

METABOLISM

PAHs are metabolized in a variety of ways, with the microsomal monooxygenases (e.g., AHH) probably most important. Steady-state activities of these enzymes vary in animals and are linked to susceptibility to some PAH-mediated cancers.[78,81] In humans, the data are much less clear. Table 7-1 summarizes the studies that suggest a correlation between high AHH inducibility (and usually high induced-AHH activity) and cancer susceptibility, and Table 7-2 summarizes the studies that suggest the converse.

Reasons for the contradictory results probably lie in methodologic variations, such as the use of different cell types, different assays, and different assay conditions. The most easily accessible and therefore commonly used human tissue is the peripheral blood lymphocytes. Nutritional state,[167] drug intake,[89] age,[35] and disease state[74] influence the capacity of the lymphocytes to respond to mitogen. These influences have not been assessed in determining their relationship to the AHH activity observed in cultured lymphocytes. Variations in AHH activity in lymphocytes have been observed to occur seasonally in some geographic locations,[128,129,154] but whether they result from in vivo or in vitro factors is not known.

A variety of in vitro conditions are known to influence AHH activity. The initial concentration of lymphocytes affects the time course and amount of control and induced AHH activity.[79] The type and lot of serum supplement infuence the control and induced AHH activity.[50,79] In fact, some lots of fetal-calf serum are capable of causing mitogen activation of lymphocytes.[205] The numbers of cultured T cells may affect the AHH activity observed.[67] In studies using cultured human tissue, two important aspects are the question of the variable degree of AHH activity in different cell types[202] and the question of large variations between and within individuals in both AHH activity and microsome-mediated BaP-DNA adduct formation.[6,53] Blood monocytes and pulmonary alveolar macrophages are examples of other human cell types whose AHH activity is correlated with that in lymphocytes[120,121] or cultured human tissue,[69] but there are problems of accessibility with each of these cell types.

If the cell samples are cultured and assayed on the same days, the variation seems to be acceptable.[50,79] Culturing lymphocytes[5,12,130] or monocytes[123] from fraternal or identical twins at the same time has shown that AHH activity is under a degree of genetic control, and the numbers of genes in question are probably small. Thus, the genetic component most likely results in a unimodal frequency distribution that is skewed in the populations of individuals toward those with higher AHH activity,[78,111] rather than the trimodal distribution originally reported.[72]

To circumvent many of the in vitro problems, the use of cryopreserved tissue may be an alternative, in that lymphocytes can be cryopreserved before mitogen activation and still have the capacity to be mitogen-activated and then assayed for AHH activity.[82] The relative AHH activities among the lymphocyte samples from different individuals are similar, whether the assays are conducted on freshly cultured lymphocytes or after cryopreservation.[82] Cryopreservation allows the culture and assay at the same time of cells from different organisms collected in diverse geographic locations and over extended periods.

The use of cryopreserved lymphocytes, control of some basic culture variables--such as initial lymphocyte concentration (1.0×10^6 cells/ml) and lot and type of serum supplement (e.g., human AB serum)--and assaying AHH activity at two times to ensure detection of peak activity can yield the data presented in Figure 7-3. Data were taken on a group of 51 persons who were on hospital diets for at least 2 d before phlebotomy, who were not on any medication, and who were eventually followed for complete clinical diagnosis. Viability of cells was measured by assay for the NADH-dependent cytochrome b_5 reductase (using cytochrome $\underline{c}$ as a substrate) activity (Cyt $\underline{c}$). Carcinogen-metabolizing activity is presented in terms of units of AHH per unit of Cyt $\underline{c}$.[79,145] The degree of mitogen activation was also measured. Data analyses showed that:

- Cryopreserved lymphocytes from over 95% of the normal and cancer patients were mitogen-activated.

- Lymphocytes from lung-cancer patients were mitogen-activated as efficiently as lymphocytes from noncancer patients (actually better; $\underline{p} = 0.001$).
- The 14 highest AHH activities were found in patients with lung cancer, with the mean in the 21 lung-cancer patients (0.89 unit AHH/unit Cyt c) being significantly higher than that in the 30 non-lung-cancer patients (0.47 unit AHH/unit Cyt c).

The higher AHH activities were not directly related to higher degrees of blastogenesis and were not related to cigarette-smoking history, tumor type, tumor location, or family history of cancer. Whether high AHH activity is the cause or the result of lung cancer cannot yet be answered.

In animal-model systems, some PAHs cause tumors of the lymphoreticular system, and a genetic association for this activity at the Ah locus has been suggested.[29,113] Although this is only presumptive, there may be a similar relationship in human leukemia patients who were recently shown to express lower AHH activity (as in animal-model systems);[11] in other studies, the first-degree relatives of leukemia patients expressed normal AHH activity.[90]

The results of these studies are interesting and certainly need to be confirmed and extended. The extended studies should be multifaceted; that is, they should simultaneously measure more than one enzymatic end point. Perhaps an appropriate group of assays would include an assay for AHH, as described in the literature; an assay for all BaP metabolites via HPLC; an assay for particulate P-450s via immunoassays;[131,158] and an assay for mRNA expression of the P-450 genes with cloned DNA fragments containing the P-450 genes.[116] Human tissues should be used where possible. There is probably a degree of genetic control of AHH activity in the human population, and this enzyme may play a role in determining susceptibility to PAH-mediated cancer and other diseases.

DNA BINDING, DAMAGE, AND REPAIR

Many PAHs are converted by the microsomal monooxygenases to forms that bind covalently to a variety of cellular macromolecules, including nucleic acids (see Chapter 5 and Phillips and Sims[140]). Evidence of the importance of DNA binding is exemplified by the observation that variations in DNA-repair capacity seem to play a major role in determining the toxic, mutagenic, and transforming activities of many chemical carcinogens, including PAHs.[3,100,149]

In animal-model systems, the amount of PAH metabolism is determined by the activity of the microsomal monooxygenases, and variations in these enzymes result in concomitant changes in the binding of chemicals to DNA.[112] In cultured human tissue, hydrocarbon-DNA binding also occurs as the result of microsomal monooxygenase-mediated metabolism,[6,55] and variations in metabolic activity are associated with concomitant variations in binding of hydrocarbons to DNA.[54] The major DNA adduct often results from the interaction of specific metabolites of PAHs (diol-epoxides) and the N7 of deoxyguanosine.[124,135] Other products are found, including interactions with the N4 deoxyadenosine,[177] the backbone phosphates of DNA,[41] and the exocyclic amino group of deoxyadenosine.[64,177] The latter may be important, because its formation from various PAH-like chemicals closely parallels their carcinogenic potencies on mouse skin.[27] No appreciable specificity of binding with respect to base sequence is apparent,[62,108,146] but binding may be influenced by chromatin structure, with a greater extent of binding associated with internucleosomal regions.[63,76]

A potentially important anomaly is that, although in vitro metabolism of BaP to forms that bind to DNA parallels the AHH activity of the microsomal preparations and the genetic background of mice used to generate these microsomal samples,[134] the in vivo results from strains of mice that differ widely in AHH activity suggest that there is very little strain variation in BaP-DNA binding.[30,139]

Probably more crucial to carcinogenicity is the geometry of the binding in relation to later excision repair by endonucleases.[48] The binding of different residues and different chemical groups within residues dramatically affects excisibility. These chemical-DNA adducts are either repaired, not repaired, or misrepaired (see Figures 7-2 and 7-4). The fate of these adducts determines whether a cell remains normal, mutates, or dies.

Repair capacity can be separated into two major types--excision repair and postreplication repair.[149] Excision repair is the in situ removal and replacement of chemically modified DNA so that the original DNA sequence is re-established. For a variety of reasons, excision-repair systems usually do not remove all the modified bases; so the DNA very often replicates, even though some unexcised damage may be present. This replicated DNA usually has gaps in the newly synthesized strand opposite the DNA adduct. The gaps are filled in by postreplication repair--also termed "recombination repair."[100] Figure 7-3 depicts how these two processes of repair contribute to the cells' survival of the damaging effects of chemicals like PAHs. A combination of both methods is involved in the repair of hydrocarbon-bound DNA.[100]

A large number of both constitutive and inducible enzymes are involved in this DNA-repair process.[47] The exact role of these enzymes is not known, but it seems that rather small changes in any of the enzyme activities can have great effects on the repair process and eventual biologic expression of the DNA adducts. Moreover, it has been recently shown in prokaryotes that the DNA adduct itself is not likely to be mutagenic, but rather that the mutagenic event is induced by the action of the DNA-repair enzymes themselves.[193]

Natural variations in DNA-repair capacity occur in humans. These variations are exemplified by the existence of genetic diseases that are associated with defects in DNA repair. Table 7-3 presents a list of such diseases, their modes of inheritance, the specific tumors associated with them, and their proposed DNA-repair defect. These genetic diseases are associated with a high incidence of malignancy, compared with the incidence in the general population, and often a specific malignancy is involved (see Table 7-3). The incidences for persons who are genetically homozygous for xeroderma pigmentosum, ataxia telangiectasia, and Fanconi's anemia are about 10^{-5}, and for those who are heterozygous, about 10^{-2}. Those who are heterozygous for ataxia telangiectasia and are less than 45 yr old have a fivefold increase in the risk of cancer,[180] and those heterozygous for Fanconi's anemia may account for 5% of all leukemia deaths (approximately a fivefold increase in susceptibility).[179] Because these people are deficient in the ability to repair radiation-induced DNA damage and chemical-induced DNA damage,[169] it has been suggested that alteration in DNA-repair capacity may put them at greater risk of chemically induced cancers.[168] It must be pointed out that many of these diseases, especially ataxia telangiectasia, are also associated with abnormalities of the immune system. Thus, genetic disease may result in higher risks of cancer via deficiencies in DNA-repair capacity or immunocompetence. Among the normal population of humans, there are probably subtle variations in DNA-repair capacity, but whether these variations are genetically controlled or are related to cancer risk remains to be determined.

PROMOTION AND COCARCINOGENESIS

Many studies have shown that a number of modifying factors can increase the effect of low-dose or low-potency carcinogens that by themselves would be insufficient to induce malignancies.[109,200] Many PAHs are complete carcinogens; that is, they have both initiating and promoting activities. Others--such as pyrene, benzo[e]pyrene, fluoranthene, and benzo[ghi]perylene--are weak complete carcinogens and weak cocarcinogens.[191,192] It is difficult to determine what role PAHs might have in tumor promotion in humans, because there are no good methods for measuring this activity in the human population. Such end points as induction of ornithine decarboxylase activity,[13] phospholipid synthesis,[159,178] inflammation,[173] protease activity,[187] cellular proliferation,[57] decrease in differentiated states,[26,201] and formation of "dark cells"[148,172] are manifestations of many promoters, and many PAHs can induce at least some of these changes.[197,200] But no single end point correlates with the promoting activity of all the different chemicals that have promoting activity.

In animal systems, there seems to be a genetic basis for promotability, in that different strains of mice express different susceptibility to promotion during the standard two-stage carcinogenesis assay. Such strains as CD-1 and BALB/c are relatively resistant, whereas the specifically derived SENCAR strain (i.e., sensitive to carcinogenesis) is very sensitive to promotion of skin cancer.[28,58] The molecular basis of this difference has not been defined, but recent information suggests that the skin itself has the sensitivity, inasmuch as skin from SENCAR mice remains sensitive to promotion even after grafting to BALB/c mice.[203]

No genetic variation in promotability in humans has been described. However, the fact that pyrenes may have promoting and cocarcinogenic activity, the possibility that such activity plays a major role in cancer formation in humans, and the absence of effective end points in the human population all suggest that much more work is necessary before the role of PAHs in promotion can be understood.

IMMUNOCOMPETENCE

Substantial interest has centered on the role of the immmune system in preventing the expression of malignancy by recognition and destruction of newly formed malignant cells. The concept of "immunosurveillance," however, has not been well supported, and, in fact, "stimulation" of malignant cells may even occur.[75]

Immunodeficient persons do have a greatly increased risk of developing a malignancy of the lymphoreticular system.[17,60,93,126] The exact mechanism responsible for the increase, however, is not clear.

A number of genetic disorders in humans are associated with immunodeficiencies. These disorders include ataxia telangiectasia, Wiskott-Aldrich syndrome, Bloom's syndrome, common variable immunodeficiency, selective IgA deficiency, Bruton's agammaglobulinemia, severe combined immunodeficiency, selective IgM deficiency, and immunodeficiency with normal or increased immunoglobulins.[73]

These immunodeficient genetic disorders are usually heterogeneously linked with a variety of other distinct underlying defects. For example, persons with ataxia telangiectasia and Bloom's syndrome have severely impaired DNA-repair capacities,[169,195] and those with severe combined immunodeficiency also have adenosine deaminase deficiency.[73] Therefore, it is difficult to determine the reasons for the increased cancer susceptibility of these persons. Epidemiologic evidence fails to support the idea that immunosurveillance mechanisms are generally involved in carcinogenesis, but does provide clues to immunologic processes that may predispose to particular neoplasms.[38]

In animal-model systems, PAHs can cause tumors of the lymphoreticular system, and association with the Ah locus has been suggested.[29,113] In humans, exposure to some hydrocarbons, such as benzene, has been repeatedly associated with leukemia. Whether variations in immunocompetence occur naturally in the normal population and whether PAHs, as a group of environmental contaminants, pose a special risk to persons with such variations are not known.

STAGE OF DEVELOPMENT

Some cell types undergo periods of heightened sensitivity to chemicals during their normal growth cycles. For example, in animal-model systems there are striking differences between germ-cell stages in the chemical induction of dominant lethals, translocations, and specific-locus mutations.[14,160] Moreover, the fetus is at greater risk than the mother, owing to high doses of environmental chemicals; the permeability of the blood-brain barrier is greater, and liver-enzyme conjugating function is poorer.[61] The greater the lipid solubility of a chemical, the greater its placental transfer; and the placenta is readily permeable to chemicals with molecular weights less than 600. Most PAHs fit into these categories, and in animal-model systems such PAHs as BaP, 3-methylcholanthrene, and 7,12-dimethylbenz[a]anthracene cause oocyte and follicle destruction and embryo lethality and resorption and have a greater incidence of malformation and even cancer in surviving embryos.[88,103,171,190]

In humans, gross congenital abnormalities occur in some 2% of all infants and are the cause of about 15% of the deaths of infants less than a year old. Exposure to such agents as viruses, mercury, DDT, CO, and polybrominated biphenyls probably accounts for 5-10% of the birth defects; genetic abnormalities cause 25%; and the causes of the remainder are largely unknown.[61] Interactions in the intrauterine environment between genetic predisposition and chemical and biologic factors are probably responsible for these birth defects. Although occupational exposure of human males[52] and both parents[204] to PAHs was not associated with increased cancer incidences in the offspring, recent work has suggested that a combination of chemical exposures of both parents (especially the mother) resulted in higher incidences of brain tumors in the offspring.[137] Maternal cigarette-smoking is associated with decreased birthweight, increased perinatal morbidity and mortality, and other harmful effects on the newborn.[141] The PAHs in cigarette smoke may account for some of its biologic activity, inasmuch as a relationship has been shown between cigarette-smoking, induction of AHH activity in human placental tissue,[114,198] and a decrease in placental size;[135] PAHs are the major class of AHH inducers found in cigarette smoke,[83] and thus it is important to note that BaP, which is in cigarette smoke, can cross the placental barrier.[92]

Because PAHs must be metabolized before they produce a biologic effect, the impact of PAHs on maternal and fetal tissues can be quite complex. Some examples of these complexities are differences in developmental patterns of specific enzymes, the relative importance of maternal and fetal metabolism, the role of metabolism in placental tissue, the relative importance of hepatic and extrahepatic metabolism, and sex differences in developmental patterns. The induced and control forms of AHH and acetanilide 4-hydroxylase are temporally regulated both before and after the birth of animals.[68] The deactivation of conjugating enzymes (e.g., UDP-glucuronyltransferase, sulfotransferase, and _N_-acetyltransferase) is also temporally regulated both before and after birth, but this regulation can be quite different from that of AHH.[94] The relation between activation and inactivation can be influenced by the sex of animals.[70] Shum _et al_.[171] showed that both the fetal and maternal enzymes play an active role in determining the ultimate fetal toxicity of BaP. Using specific crosses between AHH-responsive and AHH-nonresponsive strains, these authors could show that when the mother was nonresponsive the enzyme capacity of the fetal tissue determined the toxicity of BaP, but that when the mother was AHH-responsive there was no difference in fetal toxicity between nonresponsive and responsive fetuses. Mice seem to have AHH activity as early as about 7.5-8.5 d of gestation.[40] This activity slightly increases before birth, but increases greatly in the first few days after birth[94] and then slowly decreases as the mouse ages.[68] It should be pointed out that in vivo exposure to BaP, in addition to inducing higher AHH activity in mouse fetal tissue, can suppress humoral immunity in animals that survive and can cause about a 10-fold increase in the incidence of various tumors in surviving animals.[190] It seems likely that, in rodents (and perhaps in humans), PAHs can be taken up and distributed through the placenta intact or in the form of metabolites, that the metabolites themselves can cause fetal toxicity or the delayed effects of immune suppression or cancer, and that intact PAHs can cause fetal enzyme induction, metabolism, and the sequelae mentioned earlier.

MODIFYING FACTORS

A variety of environmental factors can mitigate or exacerbate the inherent sensitivity of mammalian tissues to PAHs. These factors are probably at least as important as some of the genetically controlled differences discussed earlier and tend to make genetic differences less distinct. Two factors known to modify PAH carcinogenesis, at least in animal-model systems, are the physical state of the PAH and the nutritional state of the exposed organism.

PHYSICAL STATE OF PAH

The sources and the formation of PAHs in the environment are discussed in Chapters 1-3. Most of them are found as mixtures and many are found in association with particles, such as cigarette-smoke particles,[174] fossil-fuel combustion products,[9] coal flyash,[77] and asbestos fibers.[86,105] This association can be important, because PAHs in the presence of or adsorbed on particles are transported through membranes more efficiently,[75] are cleared from tissue more slowly,[25] and have a different tissue distribution--that determined by the particle size, rather than by the size of the free PAH.[147] The increased uptake results in more efficient induction of AHH activity at low PAH concentrations.[105] Those exposed to particles containing PAHs are probably at greater risk of various cancers.[166] Uptake, distribution, and metabolism of PAHs can be so altered by particles that those who normally would be unaffected by the PAHs may be adversely affected.

NUTRITIONAL STATE OF HOST

Nutritional status can substantially modify the toxicity of some environmental pollutants.[99] For example, specific dietary deficiencies are known to increase the toxicity of pesticides--including carbonate carbaryl, parathion, and captan[170]--and heavy metals.[37,98] Nutritional status can influence microsomal enzymes and thus affect the toxicity of PAHs. Protein deficiencies can lower AHH activity,[101] and the type of dietary protein can affect AHH activity.[36] Nutrient deficiencies are observed in both children[125] and adults;[189] deficiencies in iron, vitamin A, and vitamin C are the most prevalent. Whether these deficiencies play a role in PAH-related effects in humans is not known. Deficiencies or alterations in vitamins (vitamins A and C) can influence the incidence of PAH-induced cancers in animal-model systems.[8,19,176] Dietary vitamin A (i.e., retinoids) may also influence the expression of cancer in humans.[138] The effects of vitamins seem to be centered on the later stages of carcinogenesis, especially tumor progression. Chemoprevention shows promise for altering or controlling inherent sensitivity (or resistance) to carcinogenesis, but it should be borne in mind that some vitamins, such as retinoids, sometimes increase cancer expression and sometimes suppress it.[164]

Diets high in fat and meat and low in fiber have been associated with increased risk of cancer, especially cancer of the colon.[51,199,200] The effect of dietary fat may be related to alterations in the concentration of colonic secondary bile acids, which act as colon-tumor promoters.[150,151] PAHs can act as cocarcinogens, comutagens, or promoters, but whether they play these roles in humans and whether the nutritional status of the host alters these roles are not known.

TABLE 7-1

Studies Suggesting Correlation between Carcinogen Metabolic Capacity and Cancer Susceptibility in Humans

Disease	Tissue Assayed	Reference	Comments
Lung cancer	Lymphocytes	72	Assay only for "inducibility"
Lung cancer	Lymphocytes	49	Radiometric assay; 11 cancer patients monitored
Laryngeal cancer	Lymphocytes	185	No controls
Lung cancer	Bronchi	53	BaP binding to DNA higher in bronchi from lung-cancer patients; large individual differences
Renal and ureteral cancer	Lymphocytes	186	No controls
Lung cancer	Lymphocytes, PAMs	106	Dichotomy of AHH in lymphocytes and PAMs in lung-cancer patients
Lung cancer	Lymphocytes	32	--
Lung cancer	Antipyrine (half-life)	1	--
Lung cancer	Lymphocytes, PAMs, lung tissue	107	Correlation of AHH depended on patient--lung cancer vs. normal
Lung cancer	Lymphocytes	39	Absolute AHH activity dominantly inherited; values given relative to "standard" panel; no AHH values presented
Lung cancer	Lymphocytes	4	Inducibility determined by non-induced AHH activity
Lung cancer	Antipyrine	71	Antipyrine half-life related to cancer and smoking
Leukemia	Lymphocytes	11	Susceptibility related to low AHH

TABLE 7-2

Studies Suggesting Lack of Correlation between Carcinogen Metabolic Capacity and Cancer Susceptibility in Humans

Disease	Tissue Assayed	Reference	Comments
Lung cancer	Lymphocytes	127	Progeny vs. spouse; cancer patients showed low AHH and were not tested
COPD, chronic bronchitis	Lymphocytes	110	--
Lung cancer	Lymphocytes	67	Smoking, not cancer, associated with high BaP metabolism; high AHH correlated with lymphocyte stimulation.
Laryngeal and lung cancer	Lymphocytes	194	Measured AHH in disease-free subjects; 40% on medication; lymphocytes from all groups grew well.
Lung cancer	Lymphocytes (BaP binding)	66	BaP-macromolecule binding measured.
Lung cancer	Lung (organ) cultures	24	HPLC analysis of BaP metabolites in six cancer patients.
Lung cancer	Antipyrine (metabolism)	188	Nine hospitalized patients used.
Lung cancer	Lymphocytes	91	--

TABLE 7-3

Human Diseases That May Be Associated with DNA-Repair Deficiencies[a]

Disease	Inheritance	Proposed Effect	Neoplastic Disease	References
Xeroderma pigmentosum, classical	Autosomal recessive, of complementation groups	Defective in excision repair of UV damage	Multiple basal-cell squamous-cell carcinomas; malignant melanoma	2,20,22,156
Xeroderma pigmentosum, variant	Autosomal recessive	Defective postreplication repair of UV damage	Multiple basal-cell squamous-cell carcinomas; malignant melanoma	21
Ataxia telangiectasia	Autosomal recessive, 2 complementation groups	Gamma-repair defect; defect in some groups in gamma-induced base damage; proposed defect in double-strand break rejoining	Lymphoma (60%), leukemia (20%), solid tumor (20%)	45,84,132,133, 165,180,195
Ataxia telangiectasia	?	Gamma-induced base damage in some cases	Gastric, gallbladder lymphoma, especially increased in patients under 45	45,84,132,133, 165,180,195
Bloom's syndrome	Autosomal recessive, especially in Ashkenazic Jews	Deflect in repair of UV damage, recombinational defect?; increased SCE in peripheral blood lymphocytes	Leukemia	18,42,43
Fanconi's anemia	Autosomal recessive	UV exonuclease deficiency?	Leukemia, squamous-cell carcinoma, mucocutaneous junctions	117,144,161,179, 181
Familial retinoblastoma	Autosomal dominant, 80-90% penetrance	Gamma repair?; defect in base-damage repair	Radiation-induced sarcomas; secondary mesenchymal tumors (mostly osteosarcoma)	34,65,196

Table 7-3 (continued)

Disease	Inheritance	Proposed Effect	Neoplastic Disease	References
D-deletion retinoblastoma	Dominant, high penetrance	Gamma-repair defect; proposed specific locus on chromosome 13	Retinoblastoma; no secondary tumors	175,195
Progeria (Hutchinson-Gilford) syndrome	Autosomal recessive	?	?	33,118,153
Down's syndrome	Autosomal recessive	Gamma-repair defect yielding excess chromosomal aberrations after ionizing radiation	Leukemia	7,87,162
Dyskeratosis congenita	X-linked recessive	Light sensitivity; excess SCE with psoralen plus light	Leukemia	16,155
Cockayne's syndrome	Autosomal recessive	Sun sensitivity; fibroblasts sensitive to UV killing	?	2,23,163
Actinia keratosis	?	UV sensitivity	Skin cancer	87
Cutaneous malignant melanoma	Dominant, ethnic clustering	Abnormal resistance to UV	Melanoma with metastasis	44,96

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[a] Data from Rasmussen[149] and Weichselbaum and Little.[195]

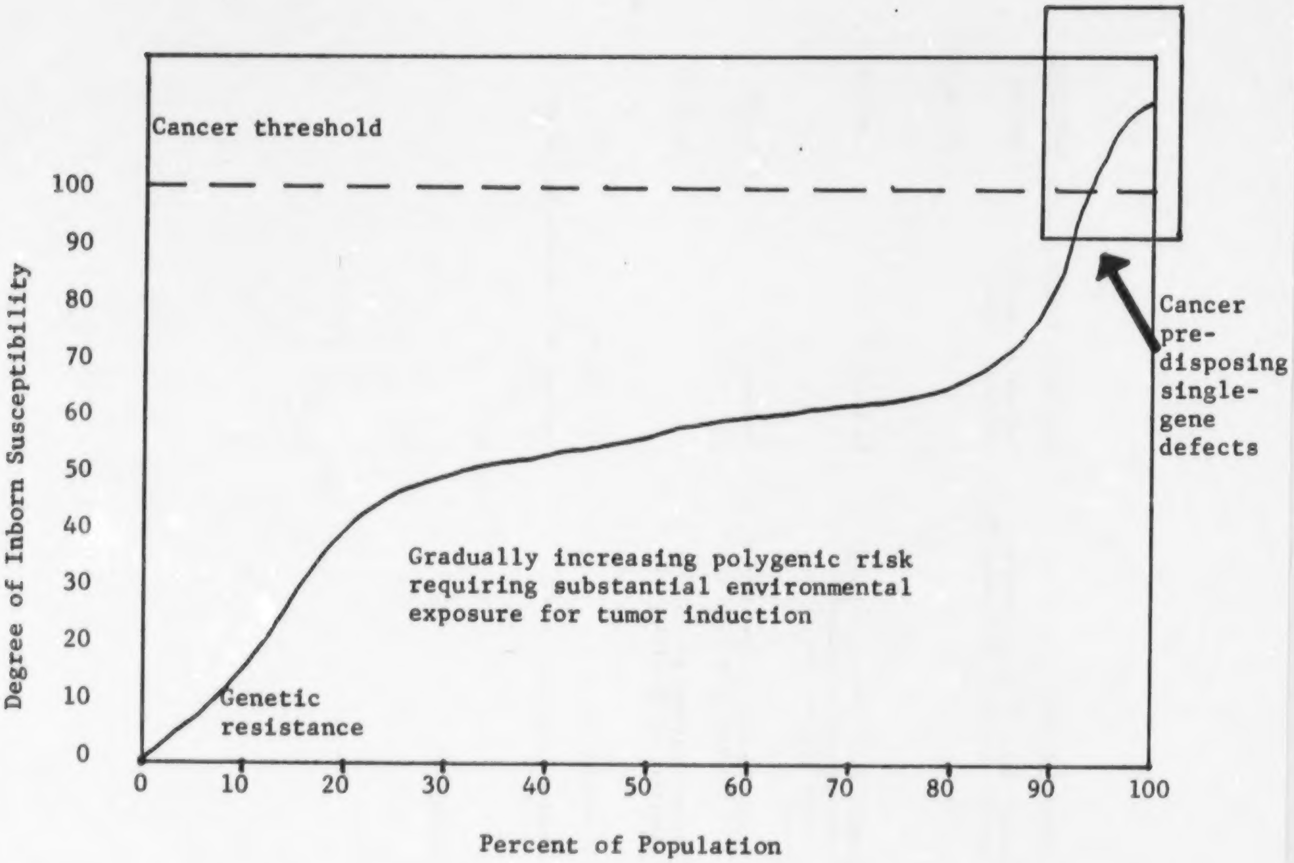

FIGURE 7-1. Interplay between genetically controlled variations and environmental exposure leading to cancer susceptibility. The population of humans is viewed as a sigmoidal curve where the extremes are either genetically resistant or genetically predisposed to cancer. The shape of the curves would be expected to change for given subpopulations that contain higher percentages of genetically resistant or genetically predisposed persons. Reprinted with permission from Lynch;[95] copyright Academic Press.

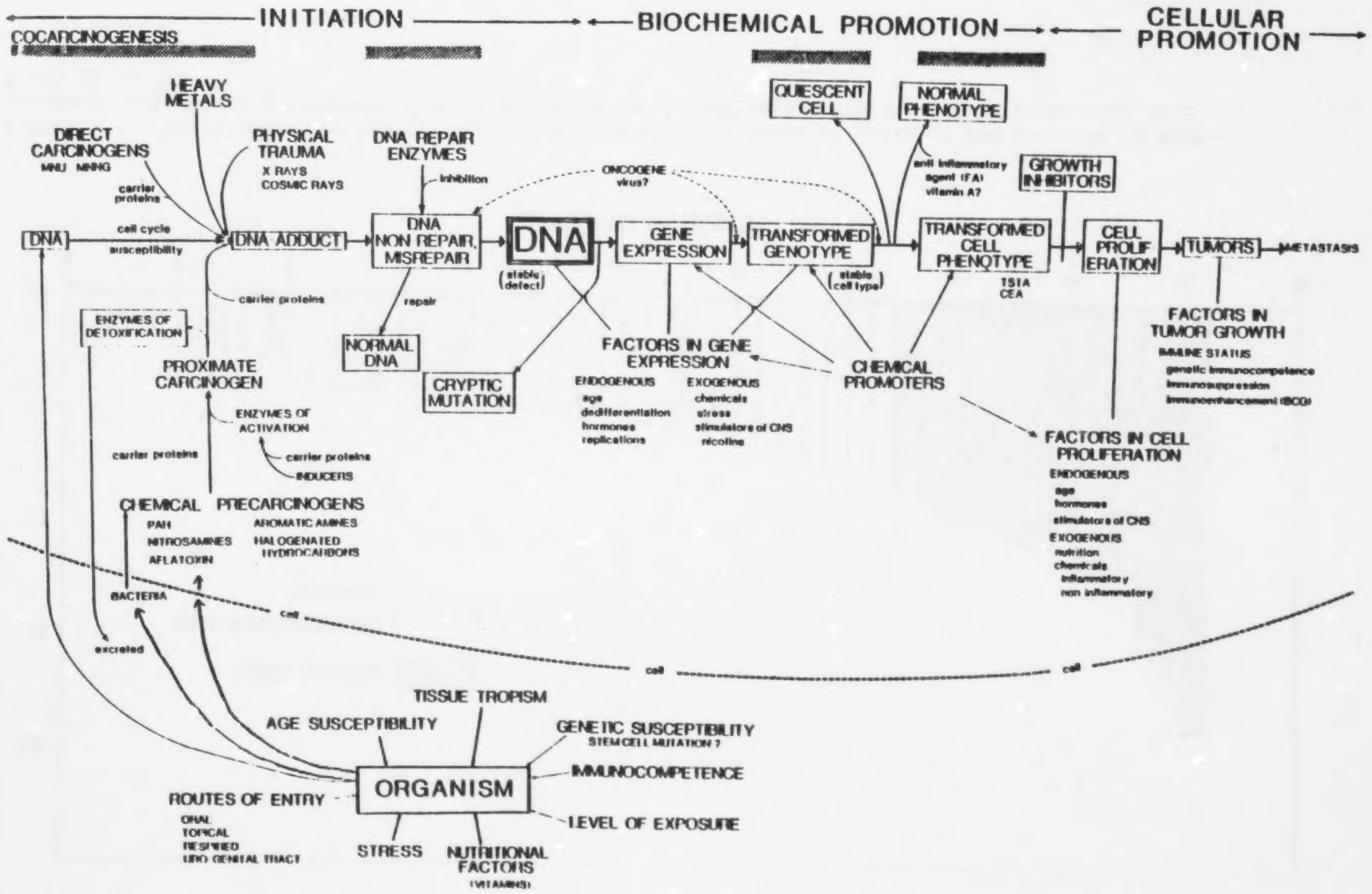
INITIATION
BIOCHEMICAL PROMOTION
CELLULAR PROMOTION
COCARCINOGENESIS
HEAVY METALS
DIRECT CARCINOGENS
MNU MNNG
carrier proteins
PHYSICAL TRAUMA
X RAYS
COSMIC RAYS
DNA REPAIR ENZYMES
inhibition
ONCOGENE virus?
QUIESCENT CELL
NORMAL PHENOTYPE
anti inflammatory agent (FA) vitamin A?
GROWTH INHIBITORS
DNA
cell cycle
susceptibility
DNA ADDUCT
DNA NON REPAIR, MISREPAIR
DNA
(stable defect)
GENE EXPRESSION
TRANSFORMED GENOTYPE
(stable cell type)
TRANSFORMED CELL PHENOTYPE
TSIA
CEA
CELL PROLIFERATION
TUMORS
METASTASIS
carrier proteins
repair
ENZYMES OF DETOXIFICATION
NORMAL DNA
CRYPTIC MUTATION
PROXIMATE CARCINOGEN
FACTORS IN GENE EXPRESSION
ENDOGENOUS
age
dedifferentiation
hormones
replications
EXOGENOUS
chemicals
stress
stimulators of CNS
nicotine
CHEMICAL PROMOTERS
FACTORS IN TUMOR GROWTH
IMMUNE STATUS
genetic immunocompetence
immunosuppression
immunoenhancement (BCG)
ENZYMES OF ACTIVATION
carrier proteins
carrier proteins
INDUCERS
FACTORS IN CELL PROLIFERATION
ENDOGENOUS
age
hormones
stimulators of CNS
EXOGENOUS
nutrition
chemicals
inflammatory
non inflammatory
CHEMICAL PRECARCINOGENS
PAH
NITROSAMINES
AFLATOXIN
AROMATIC AMINES
HALOGENATED HYDROCARBONS
BACTERIA
cell
excreted
cell
cell
TISSUE TROPISM
AGE SUSCEPTIBILITY
GENETIC SUSCEPTIBILITY
STEM CELL MUTATION ?
IMMUNOCOMPETENCE
ORGANISM
ROUTES OF ENTRY
ORAL
TOPICAL
RESPIRED
URO GENITAL TRACT
LEVEL OF EXPOSURE
STRESS
NUTRITIONAL FACTORS
(VITAMINS)

FIGURE 7-2. Hypothetical stages in chemically induced carcinogenesis. Characterization and chemical cell interactions are discussed in text. Reprinted with permission from R. E. Kouri, Genetic Differences in Chemical Carcinogenesis.[78] Copyright CRC Press, Inc., Boca Raton, Fla.

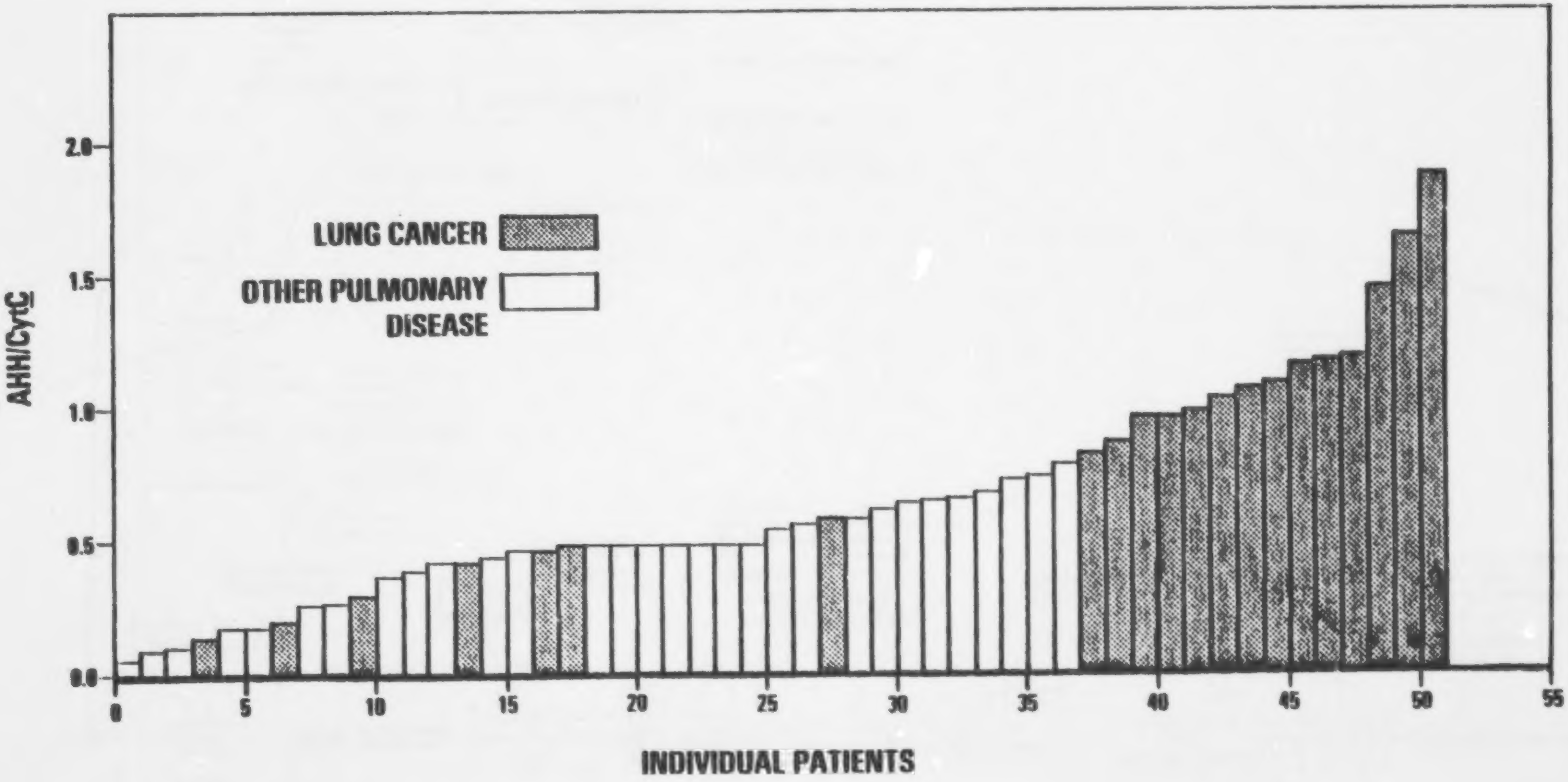

FIGURE 7-3. Distribution of hydrocarbon metabolism given in terms of units of AHH per unit of NADH-dependent cytochrome *c* reductase (cyt *c*) activity for 51 patients. Reprinted with permission from Kouri *et al.*[80]

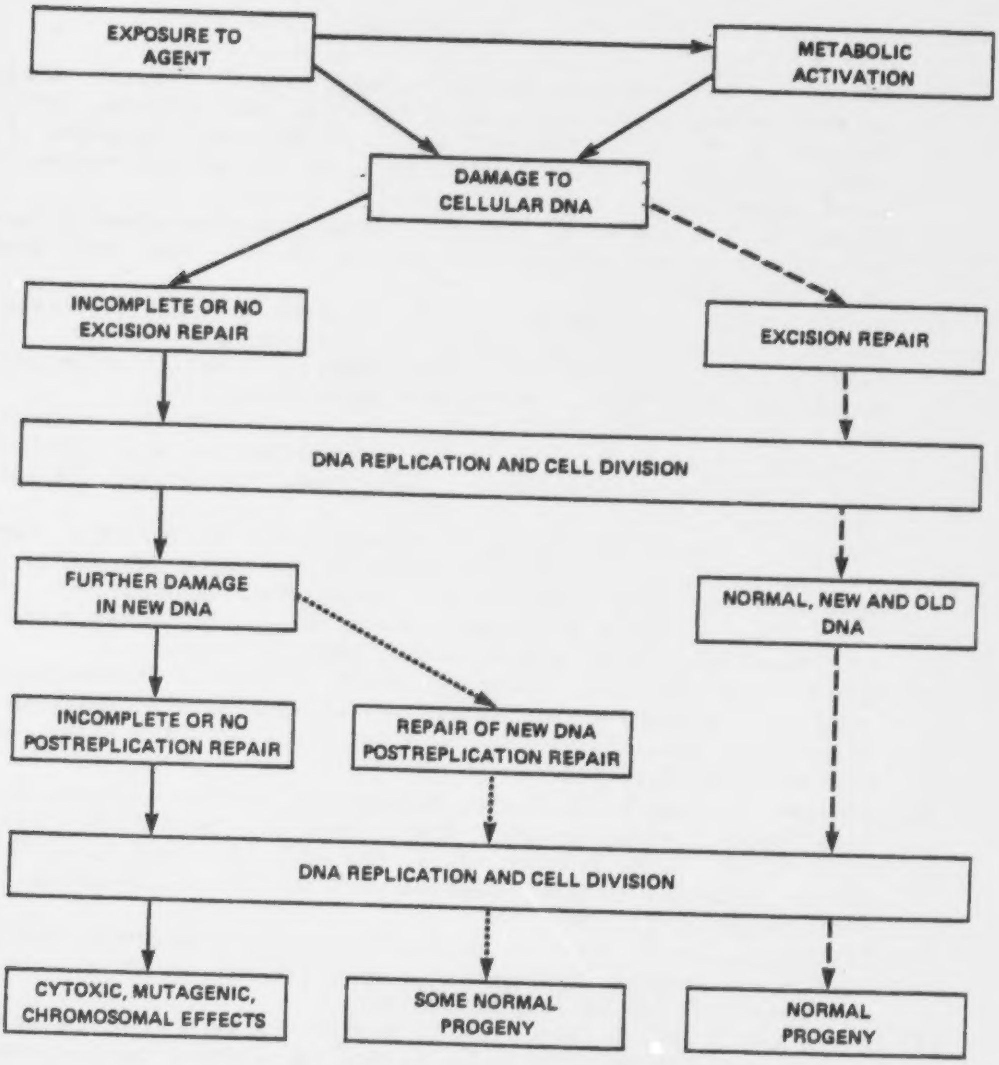
EXPOSURE TO AGENT
METABOLIC ACTIVATION
DAMAGE TO CELLULAR DNA
INCOMPLETE OR NO EXCISION REPAIR
EXCISION REPAIR
DNA REPLICATION AND CELL DIVISION
FURTHER DAMAGE IN NEW DNA
NORMAL, NEW AND OLD DNA
INCOMPLETE OR NO POSTREPLICATION REPAIR
REPAIR OF NEW DNA POSTREPLICATION REPAIR
DNA REPLICATION AND CELL DIVISION
CYTOXIC, MUTAGENIC, CHROMOSOMAL EFFECTS
SOME NORMAL PROGENY
NORMAL PROGENY

FIGURE 7-4. Scheme depicting nuclear changes and their toxic effects. Cytotoxic, mutagenic, or carcinogenic effects are thought to result from nonrepair or misrepair of particular DNA damage. Reprinted with permission from Roberts;[157] copyright Academic Press.

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8

SUMMARY

The present report attempts to make current the information relative to the sources (both mobile and stationary), formation, atmospheric transformations, biologic effects, and pharmacokinetics of a select group of polycyclic aromatic hydrocarbons (PAHs) and mixtures thereof, to identify populations hypersensitive to them and to determine the human risks associated with exposure to them. The specific PAHs considered were chosen on the basis of relative concentrations in various kinds of emission or combustion products or because of some unique pharmacologic property.

SOURCES, ATMOSPHERIC PERSISTENCE, AND TRANSFORMATIONS OF PAHs

The emphasis of this report is on PAHs emitted from mobile sources, but these substances are ubiquitous--they are found in terrestrial and aquatic plants, in soils and bottom sediments, and in fresh and marine waters, as a result of emission from both mobile and stationary sources. The total annual release of benzo[a]pyrene (BaP), as a surrogate PAH, in the United States from all sources is estimated at 300-1,300 metric tons; approximately 40 tons are produced from mobile sources. It is estimated that by the year 2000 the atmospheric BaP concentration in highly urbanized areas will be approximately 0.6 ng/m^3.

The concentration of a particular PAH depends on its source (among other things), but the phenanthrenes (including methylated derivatives), the fluorenes (including methylated derivatives), fluoranthene, pyrene, BaP, benzo[ghi]perylene, chrysene, perylene, dibenz[ac]anthracene, and benz[a]anthracene have many common sources. Emission from the combustion of wood contains more alkylated PAHs than combustion products from other sources. Wood stoves and fireplaces, nonregulated sources of PAHs, are important contributors to environmental pollution, particularly in rural areas with restricted airflow. Wood smoke contains considerable amounts of particles and adsorbed PAHs, and it is anticipated that this source will become even more significant with the increased use of wood as a primary fuel.

Of the total motor-vehicle mileage accumulated in this country, the light-duty passenger car with spark-ignition engine is the major contributor, although the number of diesel engines is increasing. By the mid-1990s, approximately 25% of the passenger fleet will probably be powered by diesel engines. Rates of emission of particles from diesel engines are about 2 orders of magnitude greater than those from catalyst-equipped spark-ignition engines. The total PAH emission from mobile sources in 1979 was approximately 6,500 metric tons; phenanthrene, pyrene, fluoranthene, methylphenanthrene, cyclopentapyrene, anthracene, benzofluorene, chrysene, benzofluoranthene, the benzopyrenes, and benzoperylene were major contributors. Nitropyrene and other nitro-PAHs have also been found as emission products, but whether these very reactive substances are artifacts of the sampling process or are present in the initial emission has not been established. It has been estimated that the total emission of PAHs in 2000 will be considerably lower because of advances in collection devices on mobile sources.

There are large uncertainties concerning the persistence of the PAHs, their chemical transformations, and their atmospheric transport and fate, although some general principles can be derived. There is evidence of long-range transport from the analysis of cores from sediments; the PAHs can be transported over long distances in the atmosphere without important degradation. The principal processes by which the PAHs are chemically removed are photooxidation, reaction with ozone, and reaction with nitrogen dioxide. The latter reaction may be responsible for the generation of nitro-PAHs, some of which are potent mutagens. Of the PAHs that have been selected for study, only BaP and pyrene have been investigated in detail with respect to chemical transformations. Considerably more study is needed.

BIOLOGIC EFFECTS OF SMOKE, EMISSION, AND SOME OF THEIR PAH COMPONENTS

It has been estimated that as much as 13% of all human cancer deaths may be attributed to environmental factors, one of which is pollution resulting from emission from mobile and stationary sources. When tested, however, particles from diesel and spark-ignition engines and organic-solvent extracts of these particles have not been very toxic to animals. Only minimal effects on pulmonary function, reproductive capacity, and glandular or hepatic function have been observed. The chronic exposure of newborn rats to diesel-engine exhaust appears to result in some abnormal development of the central nervous system, as demonstrated by the slower acquisition of spontaneous locomotor activity and bar-pressing ability; and small abnormalities have been noted in visual evoked and somatosensory evoked potentials in exposed neonatal rats. Whether these changes resulted from exposure to the PAH components of diesel-engine exhaust has not been ascertained.

Although no immunologic changes have been observed after exposure of rats to diesel-engine exhaust, it is known that some PAHs are immunosuppressive. In particular, high doses of 3-methylcholanthrene, dibenz[ah]anthracene, 7,12-dimethylbenzanthracene, and BaP reportedly depress the response of mice and rats to various immunologic challenges. This immunosuppresive effect, exhibited by some PAHs but not by exhaust or emission, can be divorced from the carcinogenicity of these agents.

Extracts of particles from spark-ignition and diesel exhaust are mutagenic to *Salmonella typhimurium* in forward- and backward-mutation assays and in several animal-cell model systems. The extracts were directly active in the bacterial assay, whereas emission from coke ovens, roofing tar, cigarette-smoke condensate, wood combustion products, and BaP were positive only after metabolic activation--indirect mutagenesis. After fractionation of the various extracts, the fraction that contained the PAHs demonstrated the greatest mutagenicity in the bacterial assay. A major PAH in soot, automobile exhaust, cigarette smoke, and coal fly ash is cyclopenta[cd]pyrene; it proved to be highly mutagenic in the indirect assay. Indeed, the total mutagenic activity of kerosene-soot extract could almost be reproduced by cyclopenta[cd]pyrene alone.

The direct mutagenicity appeared in part to be caused by nitro-PAHs. These substances have been found in automobile-exhaust particles and in cigarette smoke, but not in wood combustion products. The nitro-PAHs were much more mutagenic than the parent compounds, with 1,8-dinitropyrene being the most mutagenic of all compounds that have been subjected to the Salmonella/microsome assay. The mutagenicity of these nitro derivatives has not been tested consistently in animal-cell models.

The mouse skin tumorigenesis model has been used to assay the carcinogenicity of extracts of various particles. The condensates from spark-ignition engine exhaust proved carcinogenic in this model; those from diesel exhaust were less active. The exhaust preparations had both initiation and promotion activities with this model. There are conflicting reports as to whether the tumorigenicity of the extracts reflected the additive activity of the major PAHs in the condensates.

When tested for tumorigenicity by inhalation and intratracheal instillation, the condensates proved not very active. The literature is contradictory on whether the incidence of neoplasia in animals receiving automobile-exhaust condensate intratracheally reflected the BaP content of the condensate. Of a series of compounds that were tested for carcinogenicity in a mouse-adenoma model, 3-methylcholanthrene, dibenz[ah]anthracene, and BaP proved most active.

The effect of alkylation, particularly methylation, on the carcinogenicity of various PAHs has been determined with biologic models. The fluorenes, phenanthrenes, and anthracenes are major components of smoke and emission, so there has been considerable interest in determining the effects of methylation of these agents on tumorigenicity. The insertion of a methyl group at particular positions of the benz[a]anthracene ring increased tumorigenicity considerably. 9-Methylfluorene was much more mutagenic than the parent compound in the bacterial assay system. In the phenanthrene series, the 1- and 9-methyl analogues were more mutagenic than the parent compound. The methylchrysenes are known environmental pollutants; although the parent compound is generally inactive as a carcinogen, the 5-methyl derivative was as carcinogenic as BaP and was the most potent of all the methylated derivatives when tested as an initiator. Methylated BaPs have been tested for tumor initiation, and some (the 1-, 3-, and 11-methyl) analogues have been found to be more active in this regard than the parent compound. It is apparent that the methylated PAHs, which are present in exhaust and smoke, can contribute to carcinogenicity.

EFFECTIVE BIOLOGIC DOSE

After administration to laboratory animals, PAHs are absorbed readily and distributed to various tissues. Nonmetabolized material accumulates and persists in body fat. This phenomenon may be useful for monitoring the chronic exposure of various populations to emission and smoke that are rich in PAHs. Particle-bound PAH is retained in the lung to various degrees that depend on the size and composition of the particles. Once in the lung, the particle-bound material can be desorbed and distributed to other tissues. The clearance of a PAH from an animal-model system appears to depend on the concentration of nonmetabolized compounds in the fat, the metabolism of the PAH, and biliary, fecal, and urinary excretion. The excreted metabolites of PAH are largely glucuronides, sulfates, and hydroxylated and phenolic derivatives.

Virtually all tissues can metabolize PAHs, although liver exhibits the greatest activity in this regard. The initial metabolism is conducted by membrane-bound cytochrome P-450-dependent monooxygenases that yield epoxide derivatives. The latter may spontaneously rearrange to phenols that serve as building blocks for later conjugation. The epoxides may give rise to trans diol derivatives in reactions catalyzed by the membrane-bound enzyme epoxide hydratase; these diol derivatives may be excreted unchanged or conjugated as glucuronides. Secondary metabolism by the cytochrome P-450-dependent monooxygenases yields very reactive diol-epoxides that can spontaneously rearrange to electrophiles that can interact with macromolecular nucleophiles, such as DNA. The activity of the monooxygenases and epoxide hydratase is genetically determined and is inducible by exposure of an organism to PAHs; the extent of induction is also genetically determined.

PAHs may also be activated through an arachidonic acid-dependent co-oxygenation step involving the prostaglandin synthetase complex. Through this mechanism, the trans diol of BaP, for example, is transformed to the diol-epoxide at the expense of prostaglandin G_2.

The reactive metabolites of PAHs, such as diol-epoxides, interact covalently with DNA to form adducts. The adducts of BaP diol-epoxide with DNA have been examined in lung, liver, forestomach, colon, kidney, brain, and muscle after oral administration of BaP to mice. Human tissues also are able to catalyze adduct formation. The DNA-adduct profiles appear specific for a particular tissue. The amount of BaP-DNA adduct formed in a particular tissue is not correlated with the susceptibility of that tissue to PAH-induced carcinogenesis. This is evident from consideration of liver, a tissue that is not ordinarily a target organ for PAH-induced carcinogenesis, but one in which adducts readily form. The PAH-DNA adducts have varied turnover rates in different tissues. The turnover rate is related in part to the normal rate of replication of the cell and in part to an enzymatic DNA-repair system. Different adducts are removed from DNA at different rates.

With regard to BaP, a linear dose-response relationship has been observed with formation of DNA adducts as the end point. There appears to be no threshold dose below which adduct formation will not occur. The administration of a number of inducers of monooxygenases and of the conjugating enzyme systems reduces the in vivo formation of adducts; administration of antioxidants has a similar effect. It has been proposed that the concentration of PAH-DNA adducts in a particular tissue can be used as a measure of the "effective biologic dose" of a specific PAH. It should be simple to determine this dose with currently available sensitive radioimmunoassay methods. Such methods could be applied to readily accessible lymphocytes of human populations.

HUMAN EXPOSURE TO AND METABOLISM OF PAHs

Humans are exposed to PAHs almost exclusively through the gastrointestinal and respiratory tracts. Possibly 99% of exposure to these substances is through the diet. The daily human exposure to PAHs from air, water, and food has been estimated. Of approximately 1.8-16 μg of total PAHs ingested or inhaled, 0.2 and 0.02 μg would be derived from inhalation or ingestion in water, respectively, and the rest from food. Of the total, approximately 10% would be BaP.

Although the PAHs are ubiquitous in foodstuffs, their content can be surprisingly high in some foods as a result of pollution from soils, irrigation waters, atmospheric fallout, and food-processing. The number of PAHs ingested may be as high as 100, or even higher. Boiling or barbecuing substantially affects the composition and quantity of PAHs in foods.

Occupational exposure to PAHs can lead to inhalation of great quantities. It has been estimated that a normal adult breathing 20 m^3 of air per day can inhale approximately 700 μg of PAHs per day in a work setting that is rich in PAHs, e.g., coal and pitch-coking plants, gasworks, and roof-tarring operations. It has also been estimated that people who remain in tunnels with heavy motor traffic all day can inhale BaP that would be equivalent to that found in a pack of "old-style" cigarettes. In accordance with the occupational exposure, cancer mortality among men employed in coal-tar industries reflects excess cancer in one or more sites, particularly those involving the lungs.

The manner by which PAHs gain access to the systemic circulation is not known. Serum lipoproteins may constitute a substantial circulatory pool of the PAHs, which can be transferred into cells by a non-receptor-mediated process. The pharmacokinetics of PAHs other than BaP in humans are not well understood.

Normal and malignant human tissues have the metabolic capacity to biotransform PAHs, especially BaP. The individual variation in this capacity is very large in the human and appears to be genetically determined. Although it has been proposed that aryl hydrocarbon hydroxylase activity in lymphocytes and monocytes of lung-cancer patients is highly inducible, compared with that in "normal" patients, this relationship has not been definitively established and deserves further study.

There is little information to implicate diet-derived PAHs in any form of clinical pathology, despite the relatively large amounts of these compounds ingested. The gastrointestinal system, including the liver, may be relatively "resistant" to the PAHs; the nature of such resistance should be explored.

POPULATIONS OF HYPERSENSITIVE PERSONS

The exposure of cells or animals to pollutants, including PAHs, can lead to toxicoses, mutagenesis, carcinogenesis, and teratogenesis. Susceptibility to PAH-induced effects may be controlled at the level of uptake into specific cells, metabolic activation or inactivation, DNA repair, expression of DNA damage and its progression to the phenotype of a mutant cell, and immunocompetence of the person. Several of these steps (perhaps all) are subject to genetic regulation, although information in this regard is sketchy. Natural variations in capacity for human DNA repair lead to increased susceptibility to cancer in some instances, but the role of the PAHs in this development is not established. Genetically controlled variations in immunocompetence are observed in people with high susceptibility to carcinogenesis; no definitve role of the PAHs has been suggested. The physical state of a PAH and the nutritional or developmental state of the host contribute substantially to the observed biologic effect.

9

RECOMMENDATIONS

MOBILE SOURCES

Several of the polycyclic aromatic hydrocarbons (PAHs) found in emission from heavy-duty diesel vehicles and other vehicles are potentially hazardous to human health. On the basis of what is currently known, research should be conducted to discover practical and economical adjustments in engine design for reducing particulate and gaseous PAH emission. In vitro mutagenesis tests could be used to determine the types of adjustments that influence the concentrations of PAH chemicals active in these short-term tests. On preliminary testing, the nitro-PAHs have been mutagenic; thus, they are an important subgroup of the PAHs purportedly found in mobile-source emission. However, it is not clear whether these compounds are formed in exhaust or are artifacts of sampling; more information is needed to clarify this issue.

ATMOSPHERE

Data from core sampling of bottom sediments in rivers and bays show long-range transport of presumably unreacted PAHs. PAH chemistry of urban and industrial emission plumes should be systematically studied both regionally and on a continental scale.

It is recommended that monitoring of wet and dry PAH deposition be included in existing ambient-air quality monitoring networks. The heterogeneous photooxidation and reactions of PAH with ozone and oxides of nitrogen should be examined under experimental conditions with emphasis on the nature and size distribution of carrier particles on both PAH and reaction products; the findings should be correlated with findings on what actually occurs in the ambient air.

A system for monitoring in large residential localities should be encouraged, to determine the concentrations of PAHs emitted from residential fireplaces, wood-burning stoves, and coal-fired heating systems and the contributions from these sources relative to those from industrial and commercial boilers and rural municipal waste-burning units.

Concurrently with the monitoring studies, research should be conducted on design of equipment, technologies, or methods for controlling PAH emission from residential fireplaces and wood- and coal-burning stoves. Extracts of the condensates of smoke and other gaseous emission from wood, coal, diesel and spark-ignition engines, and tobacco must continue to be tested in in vitro mutagenicity systems, so that activity profiles can be established and specific active PAHs identified. There is a need to develop double checks on the findings of research on extracts of condensates, to eliminate the uncertainty regarding artifacts that occur in the sampling or extraction processes. The mutagenicity and carcinogenicity of each active PAH (especially nitro-PAHs and sulfur-containing PAHs) should be determined in several animal-model systems to guide the assessment of their contribution to human disease.

EXPERIMENTAL-ANIMAL STUDIES

Some data on cocarcinogenic activity of PAHs with other chemicals are available, but this data base needs to be strengthened, and PAHs other than benzo[a]pyrene (BaP) need to be studied further. Specifically, data are needed to establish whether various PAHs exhibit cocarcinogenic activity with other components of exhaust from mobile sources or emission from other combustion sources, especially wood smoke. The potential promoting activity of PAHs (including BaP) needs to be established. A model for promotion other than the mouse skin tumorigenesis system is needed. Of special interest would be a promotion system using human cells.

Extrapolation of findings from animal studies to humans is tentative without additional biochemical and pharmacokinetic data. Sorting out the toxic chemicals in any complex mixture (such as automobile exhaust, wood smoke, or cigarette smoke) is always difficult. Animal models and compound-specific testing systems are needed to ascertain the toxic effects (if any) of long-term (chronic) exposure of animals to diesel exhaust and other complex kinds of emission. In this regard, it is important to stress that the animal model systems include introduction of the PAHs (alone, in mixtures, and bound to particles) into the diets of animals in lifetime studies of carcinogenesis. Such dietary exposure is based on the data that indicate that ingestion contributes heavily to the body burden of the PAHs. As results from these studies begin to distinguish the toxic components, biochemical and pharmacokinetic data on experimental primates (e.g., squirrel monkeys) will be particularly useful in confirming the findings in animal species and extrapolating to humans. With improving characterization of the toxic components, studies should be conducted on lung deposition, uptake, and clearance of PAHs. Studies on the relationships of carrier-particle size, surface properties in the submicrometer range, and absorption and adsorption of individual PAHs should be continued and expanded with an eye to learning the source of the greatest exposure to the toxic chemicals.

Preliminary studies in animal models should be conducted as soon as possible to determine the relationship of PAH exposure to birth defects and other genetic anomalies. Specifically, it would be important to know whether chronic exposure of newborns to various types of exhaust and smoke and to mixtures of PAHs and individual PAHs (present in high concentrations in exhaust) affects development of the central nervous system.

DNA ADDUCTS, ENZYME INDUCERS, AND REPAIR

What is the relationship of the enzymes and their activity to the metabolism of PAHs, other than BaP, and to the formation of PAH-DNA adducts and their repair? A broader question is: What are the consequences of the various DNA adducts known to be formed?

To answer these questions, more sensitive and specific assays must be developed for detecting PAH metabolite-DNA adducts, e.g., with monoclonal antibodies. Such assays would be used to determine rates of PAH metabolite-DNA adduct formation in individual cell types and in organs, such as the lung, after in vivo experimental exposure to PAHs, especially low-dose, long-term exposure. With appropriately designed cell-model systems that use various cell types, the relationship of in vivo repair of PAH metabolite-DNA adducts should be examined and an activity profile developed for the individual known active PAHs. Animals other than mice and rats should be used to examine PAH metabolite-DNA adduct formation and the mechanisms by which phenolic antioxidants and inducers of aryl hydrocarbon hydroxylase (AHH) inhibit the formation of adducts.

Can the PAH metabolite-DNA systems be quantified and further developed for use in monitoring exposure to specific PAHs? The feasibility of using adducts as a measure of effective biologic dose should be studied for low-dose extrapolation of bioassay findings to dose-response curves that show the rate of adduct formation and its relationship to PAH-induced neoplasia in animal-model systems. The importance of the findings will depend on a careful analysis of the background concentrations of PAH-DNA adducts in tissues--i.e., "noise."

HUMAN STUDIES

Obviously, all health-related research findings are useful in improving the protection of human health. Although research that uses human beings directly poses difficult problems, there are various kinds of human studies that avoid those problems. For instance, human tissues can be used to study the relationship of specific biotransformations of PAHs to findings of carcinogenicity in animals.

To determine the PAH dose absorbed from human lung tissue, there is a need to know the chemical form and binding of PAHs on particles, particle size, composition, clearance rates, and ultimate fate of inhaled particle-adsorbed PAHs. These findings would be essential in studying the relationship of formation of PAH metabolite-DNA adducts and the incidences of adverse health effects found in animal studies.

Progress in understanding research findings could be greatly improved if an "inventory" of PAHs identified and measured in normal and diseased human tissues could be developed. Perhaps samples of appropriate tissues could be analyzed specifically for this purpose, and biologic and historical information on the donors could be accumulated. The tissue profiles of PAH metabolite-DNA adducts or other indicators could be compared with those derived from environmental sampling or air monitoring.

The findings in this report show that a high fraction of human exposure to PAHs is attributable to dietary intake. The possible relationship of ingested PAHs to increased incidences of gastrointestinal (or other) malignancies should be included in epidemiologic analyses.

Such analyses should attempt to isolate the portion of the prevailing gastrointestinal malignancy rate in selected populations that is due to food-derived exposure to PAHs. It is apparent that there is resistance in the gastrointestinal system to the carcinogenic potential of the PAHs. The mechanisms responsible for this resistance might involve a great variety of body systems; no specific body function can be pinpointed. However, some effort should be directed toward finding these mechanisms. There can be few clinical parallels to this combination of (1) sustained impingement of carcinogenic compounds on a system of tissues and (2) so little evidence of realization of the potential deleterious effects of such chemicals as the PAHs.

The following studies are suggested for the further development and evaluation of models for assessing the carcinogenicity relationships in humans or cell cultures derived from humans.

- Consider the use of radiolabeled tracers or immunologic methods to study the metabolism of select PAHs, such as benzo[a]pyrene, in humans. The absolute amounts of compound required for single-dose exposure would be insignificant, compared with the heavy daily exposure commonly found in foods, but the medical and scientific value of the data obtained would be very large indeed.
- Examine the metabolism, pharmacokinetics, and DNA binding of nitro-PAHs.
- Conduct systematic studies of the patterns of tissue enzymatic activities relevant to PAH metabolism as a function of age, sex, hormone activities, nutritional state, or state of health (disease).
- Correlate enzymatic activities, especially those involved in PAH activation to ultimate carcinogens, in one tissue type with the same biochemical properties of other tissues in the same person. These data would have the great advantage of eliminating the factor of genetic diversity in assessing the pathophysiologic significance of such enzymatic characteristics.
- Determine which genetically controlled deficiencies in immunocompetence are related to specific immune dysfunctions.
- Develop better methods for determining the numbers of heterozygotes at any given locus and use these methods specifically in populations exposed to high concentrations of PAHs.
- On the basis of such data, monitor the development of DNA adducts in humans with the hope of extrapolating to cancer risk.
- Reassess the role of genetically mediated differences in AHH responsiveness in determining cancer susceptibility by using multiple human tissues and multiple enzyme end points (assay for PAH receptors in human tissue; assay for total and specific cytochrome P-450s by monoclononal antibodies; assay for AHH expression of these genes; use of lymphoid, epidermal, and fibroblastic cells as sources of tissues for enzymatic assays; and use of multiple functional assays for AHH, e.g., fluorimetry, high-performance liquid chromatography, and DNA binding and repair).
- Determine whether the promotion-associated steps that occur in mouse skin also occur in human skin. Attempt to develop assays to measure for "promotability" among humans; i.e., are there genetic variants among humans for "promotability"?

- Undertake occupational studies of persons exposed to high concentrations of PAHs. These studies would record detailed information on job histories and smoking habits of all persons studied, so that the effects attributable to occupational PAH exposure and cigarette-smoking could be assessed.
- Study the relationship of PAH measurements to the various defined job categories. A studied control group (non-PAH-exposed) must be included.

APPENDIX A

LISTS OF POLYCYCLIC AROMATIC HYDROCARBONS

This appendix consists of four tables. The first is an alphabetical list of polycyclic aromatic hydrocarbons (PAHs) discussed in the report and close chemical relatives, with molecular formulas and CAS numbers. The second is a list of structural formulas (ordered according to structural complexity) and ratings of carcinogenic activity; these ratings indicate only relative activity. The third table lists nitroarenes that have been detected in particulate extracts of diesel exhaust, and the fourth shows their structural formulas.

TABLE A-1

Polycyclic Aromatic Hydrocarbons and Related Compounds: Molecular Formulas and CAS Numbers

Name	Molecular Formula	CAS No.
Acenaphthylene	$C_{12}H_8$	208-96-8
Acephenanthrylene	$C_{16}H_{10}$	201-06-9
Acridine	$C_{13}H_9N$	260-94-6
Anthanthrene	*see* Dibenzo[def,mno]chrysene	
Anthracene	$C_{14}H_{10}$	120-12-7
9,10-Anthracenedione	$C_{14}H_8O_2$	84-65-1
9(10*H*)-Anthracenone	$C_{14}H_{10}O$	90-44-8
Anthraquinone	*see* 9,10-Anthracenedione	
Anthrone	*see* 9(10*H*)-Anthracenone	
Benz[e]acephenanthrylene	$C_{20}H_{12}$	205-99-2
Benz[c]acridine	$C_{17}H_{11}$	225-51-4
Benz[a]anthracene	$C_{18}H_{12}$	56-55-3
7*H*-Benz[de]anthracen-7-one	$C_{17}H_{10}O$	82-05-3
Benzanthrone	*see* 7*H*-Benz[de]anthracen-7-one	
Benzo[b]chrysene	$C_{22}H_{14}$	214-17-5
Benzo[c]chrysene	$C_{22}H_{14}$	194-69-4
Benzo[g]chrysene	$C_{22}H_{14}$	196-78-1
Benzo[c]cinnoline	$C_{12}H_8N_2$	230-17-1
Benzo[a]dibenzothiophene	*see* Benzo[b]naphtho[2,1-d]-thiophene	
Benzo[b]fluoranthene	*see* Benz[e]acephenanthrylene	
Benzo[ghi]fluoranthene	$C_{18}H_{10}$	203-12-3
Benzo[j]fluoranthene	$C_{20}H_{12}$	205-82-3
Benzo[k]fluoranthene	$C_{20}H_{12}$	207-08-9
11*H*-Benzo[a]fluorene	$C_{17}H_{12}$	238-84-6
11*H*-Benzo[b]fluorene	$C_{17}H_{12}$	243-17-4
7*H*-Benzo[c]fluorene	$C_{17}H_{12}$	205-12-9
Benzo[h]naphtho[1,2-f]quinolene	$C_{21}H_{13}$	196-79-2
Benzo[b]naphtho[2,1-d]thiophene	$C_{16}H_{10}$	239-35-0
Benzo[rst]pentaphene	$C_{24}H_{14}$	189-55-9
Benzo[ghi]perylene	$C_{22}H_{12}$	191-24-2
Benzo[c]phenanthrene	$C_{18}H_{12}$	195-19-7
Benzo[a]pyrene	$C_{20}H_{12}$	50-32-8
Benzo[e]pyrene	$C_{20}H_{12}$	192-97-2
Benzo[f]quinoline	$C_{13}H_9$	85-02-9
Benzo[h]quinoline	$C_{13}H_9$	230-27-3
Benzo[b]triphenylene	$C_{22}H_{14}$	215-58-7
Biphenylene	$C_{12}H_8$	259-79-0
9*H*-Carbazole	$C_{12}H_9$	86-74-8
Chrysene	$C_{18}H_{12}$	218-01-9
Coronene	$C_{24}H_{12}$	191-07-1
4*H*-Cyclopenta[def]phenanthrene	$C_{15}H_{10}$	203-64-5
Cyclopenta[cd]pyrene	$C_{18}H_{10}$	27208-37-3
Dibenz[a,h]acridine	$C_{21}H_{13}$	226-36-8
Dibenz[a,j]acridine	$C_{21}H_{13}$	224-42-0
Dibenz[c,h]acridine	$C_{21}H_{13}$	224-53-3

Table A-1 (continued)

Name	Molecular Formula	CAS No.
Dibenz[a,c]anthracene	see Benzo[b]triphenylene	
Dibenz[a,h]anthracene	$C_{22}H_{14}$	53-07-3
Dibenz[a,j]anthracene	$C_{22}H_{14}$	224-41-9
7H-Dibenzo[a,g]carbazole	$C_{20}H_{13}$	207-84-1
13H-Dibenzo[a,i]carbazole	$C_{20}H_{13}$	239-64-5
7H-Dibenzo[c,g]carbazole	$C_{20}H_{13}$	194-59-2
Dibenzo[b,def]chrysene	$C_{24}H_{14}$	189-64-0
Dibenzo[def,mno]chrysene	$C_{22}H_{12}$	191-26-4
Dibenzo[def,p]chrysene	$C_{24}H_{14}$	191-30-0
Dibenzo[b,h]phenanthrene	see Pentaphene	
Dibenzo[a,e]pyrene	see Naphtho[1,2,3,4-def]chrysene	
Dibenzo[a,h]pyrene	see Dibenzo[b,def]chrysene	
Dibenzo[a,i]pyrene	see Benzo[rst]pentaphene	
Dibenzo[a,l]pyrene	see Dibenzo[def,p]chrysene	
Dibenzothiophene	$C_{12}H_8S$	132-65-0
Fluoranthene	$C_{16}H_{10}$	206-44-0
9H-Fluorene	$C_{13}H_{10}$	86-73-7
9H-Fluoren-9-one	$C_{13}H_8O$	484-25-9
Indeno[1,2,3-cd]pyrene	$C_{22}H_{12}$	193-39-5
1H-Indole	C_8H_7N	120-72-9
Isoquinoline	C_9H_7N	119-65-3
Naphthacene	$C_{18}H_{12}$	92-24-0
Naphthalene	$C_{10}H_8$	91-20-3
Naphtho[1,2,3,4-def]chrysene	$C_{24}H_{14}$	192-65-4
Naphtho[2,3-f]quinoline	$C_{17}H_{11}$	224-98-6
Pentaphene	$C_{22}H_{14}$	222-93-5
Perylene	$C_{20}H_{12}$	198-55-0
1H-Phenalene	$C_{13}H_{10}$	203-80-5
Phenanthraquinone	see 9,10-Phenanthrenedione	
Phenanthrene	$C_{14}H_{10}$	85-01-8
9,10-Phenanthrenedione	$C_{14}H_8O_2$	84-11-7
Phenanthridine	$C_{13}H_9N$	229-87-8
1,10-Phenanthroline	$C_{12}H_8N_2$	66-71-7
Phenanthro[4,5-bcd]thiophene	$C_{14}H_8S$	30796-92-0
Phenazine	$C_{12}H_8N_2$	92-82-0
Phenazone	see Benzo[c]cinnoline	
Picene	$C_{22}H_{14}$	213-46-7
Pyrene	$C_{16}H_{10}$	129-00-0
Quinoline	C_9H_7N	91-22-5
Triphenylene	$C_{18}H_{12}$	217-59-4
9H-Xanthene	$C_{13}H_{10}O$	92-83-1

TABLE A-2

Polycyclic Aromatic Hydrocarbons and Related Compounds: Structural Formulas, Molecular Weights, and Carcinogenic Activity

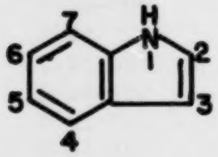
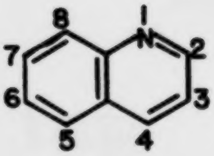
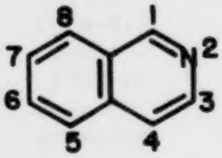
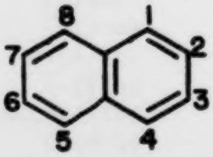
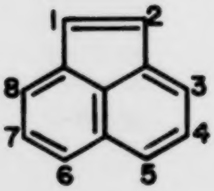
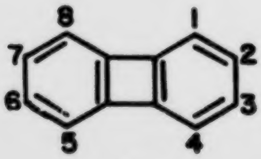
Structural Formula	Name	Molecular Weight	Carcinogenic Activity[a]
	1H-Indole	117.0578	0
	Quinoline	129.0578	+
	Isoquinoline	129.0578	0
	Naphthalene	128.0626	0
	Acenaphthylene	152.0626	0
	Biphenylene	152.0626	NA

Table A-2 (continued)

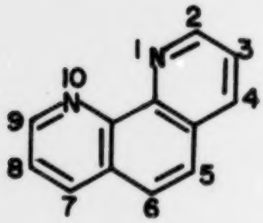
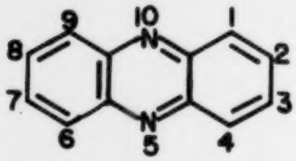
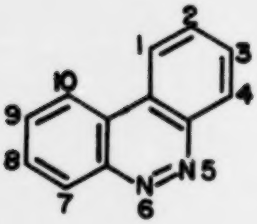
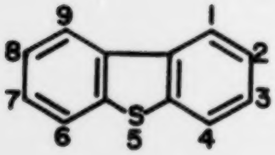
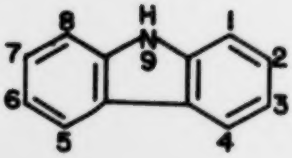
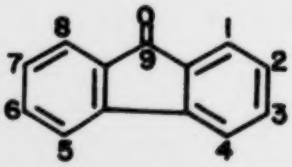
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
 1 N 2 3 4 5 6 7 8 9 10 N	1,10-Phenanthroline	180.0687	NA
 9 10 N 1 8 2 7 3 6 N 5 4	Phenazine	180.0687	NA
 1 2 3 10 4 9 8 N 5 N 6 7	(Phenazone) Benzo[c]cinnoline	180.0687	NA
 9 1 8 2 7 3 6 S 5 4	Dibenzothiophene	184.0347	0
 8 H N 9 1 7 2 6 3 5 4	9H-Carbazole	167.0735	0
 O 9 8 1 7 2 6 3 5 4	9H-Fluoren-9-one	180.0575	NA

Table A-2 (continued)

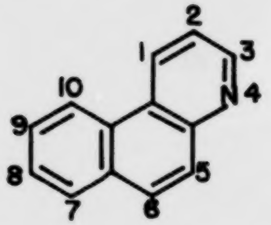
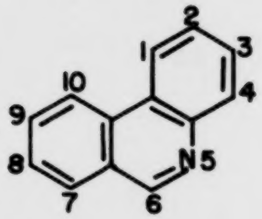
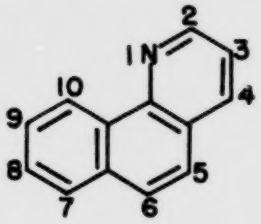
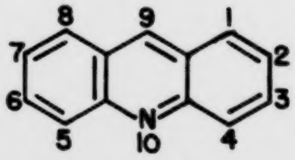
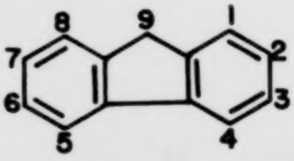
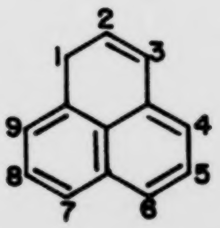
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
	Benzo[f]quinoline	179.0735	0
	Phenanthridine	179.0735	+
	Benzo[h]quinoline	179.0735	0
	Acridine	179.0735	0
	9H-Fluorene	166.0783	0
	1H-Phenalene	166.0783	NA

Table A-2 (continued)

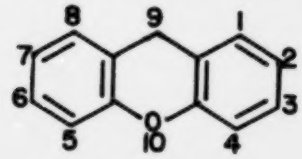
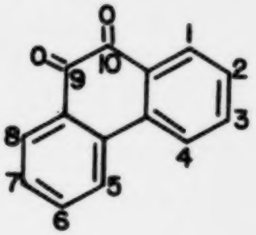
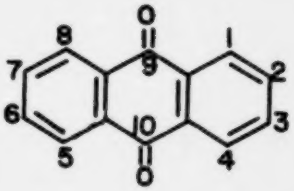
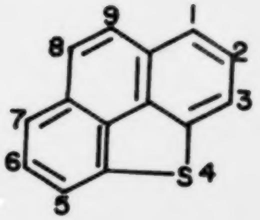
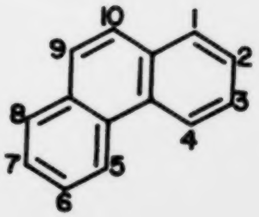
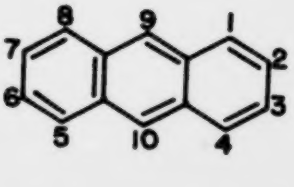
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
	9$\underline{H}$-Xanthene	182.0732	NA
	9,10-Phenanthrenedione (Phenanthraquinone)	208.0524	NA
	9,10-Anthracenedione (Anthraquinone)	208.0524	NA
	Phenanthro[4,5-bcd]-thiophene	208.0347	NA
	Phenanthrene	178.0783	0
	Anthracene	178.0783	0

Table A-2 (continued)

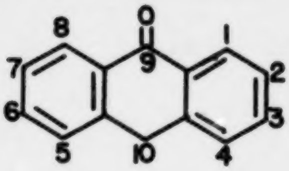
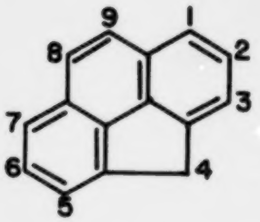
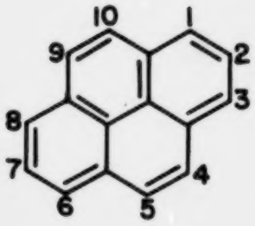
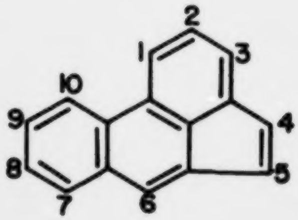
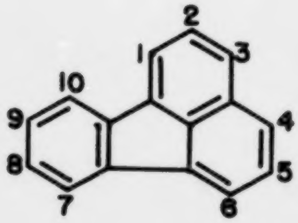
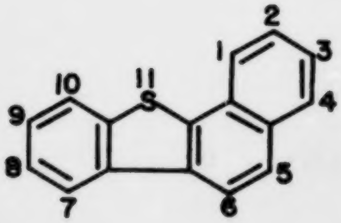
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
	9[10H]-Anthracenone (Anthrone)	194.0732	0
	4H-Cyclopenta[def]-phenanthrene	190.0783	NA
	Pyrene	202.0783	0
	Acephenanthrylene	202.0783	NA
	Fluoranthene	202.0783	+
	Benzo[b]naphtho-[2,1-d]thiophene (Benzo[a]dibenzothiophene)	234.0503	0

Table A-2 (continued)

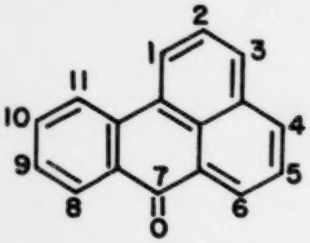
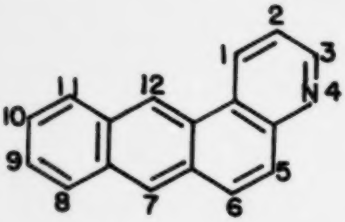
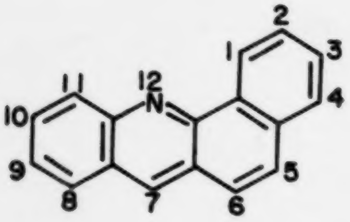
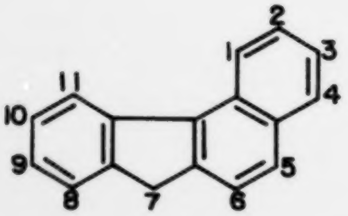
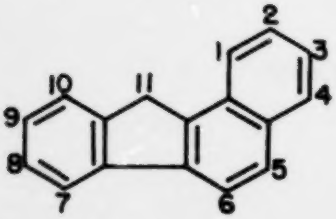
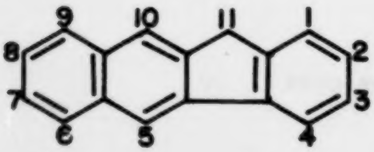
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
	7_H_-Benz[de]anthracen-7-one (Benzanthrone)	230.0732	NA
	Naphtho[2,3-f]quinoline	229.0891	0/+
	Benz[c]acridine	229.0891	+
	7_H_-Benzo[c]fluorene	216.0939	0
	11_H_-Benzo[a]fluorene	216.0939	0
	11_H_-Benzo[b]fluorene	216.0939	NA

Table A-2 (continued)

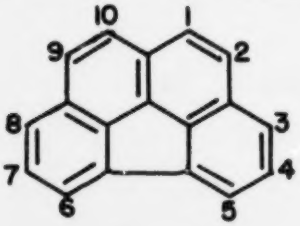
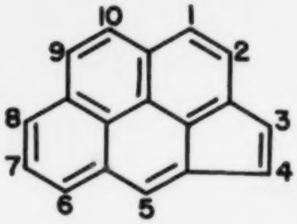
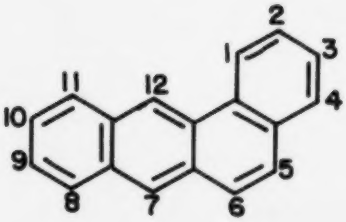
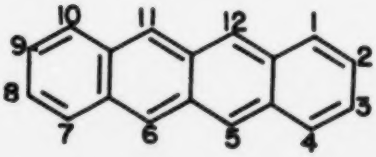
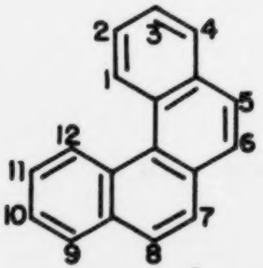
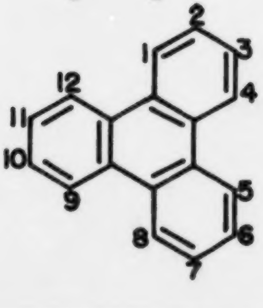
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
 10 1 2 3 4 5 6 7 8 9	Benzo[ghi]fluoranthene	226.0783	0
 10 1 2 3 4 5 6 7 8 9	Cyclopenta[cd]pyrene	226.0783	+
 2 1 3 4 11 12 10 9 5 8 7 6	Benz[a]anthracene	228.0939	+
 10 11 12 1 9 2 8 3 7 6 5 4	Naphthacene	228.0939	0
 2 3 4 1 5 12 6 11 10 7 9 8	Benzo[c]phenanthrene	228.0939	+
 2 1 3 12 4 11 10 5 9 8 6 7	Triphenylene	228.0939	0

Table A-2 (continued)

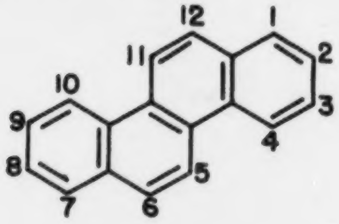
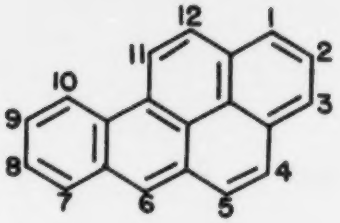
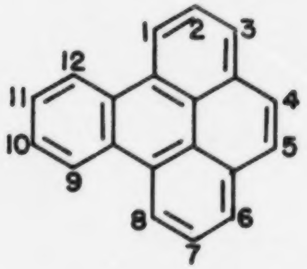
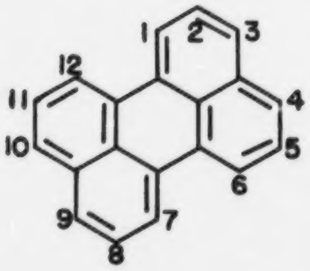
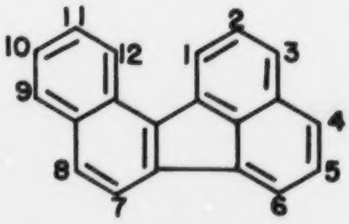
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
	Chrysene	228.0939	0/+
	Benzo[a]pyrene	252.0939	++
	Benz[e]pyrene	252.0939	0/+
	Perylene	252.0939	0
	Benzo[j]fluoranthene	252.0939	++

Table A-2 (continued)

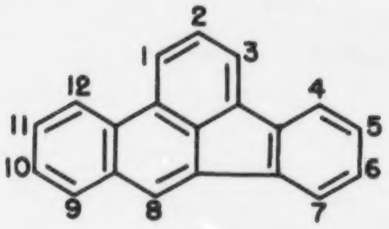
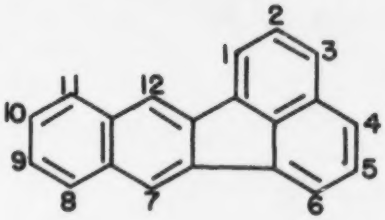
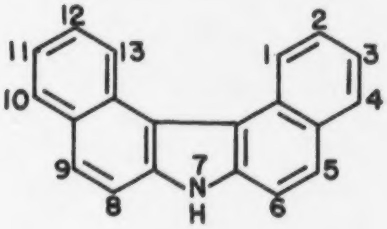
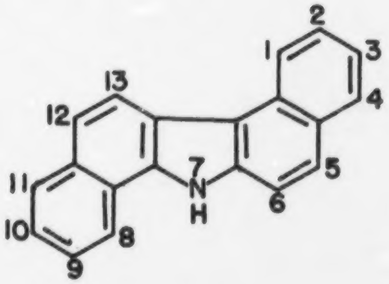
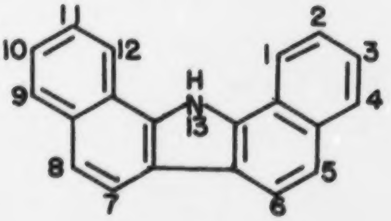
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
	Benz[e]acephenanthrylene (Benzo[b]fluoranthene	252.0939	++
	Benzo[k]fluoranthene	252.0939	++
	7H-Dibenzo[c,g]carbazole	267.1048	++
	7H-Dibenzo[a,g]carbazole	267.1048	+
	13H-Dibenzo[a,i]carbazole	267.1048	+

Table A-2 (continued)

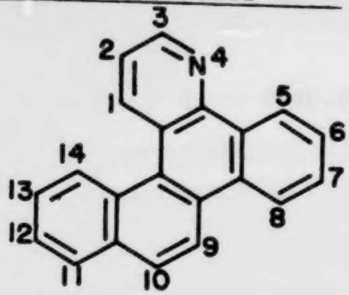
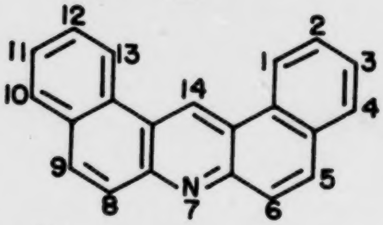
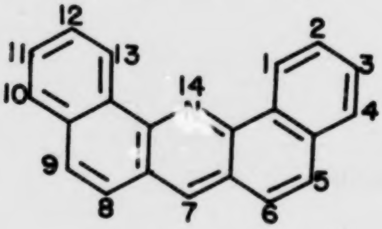
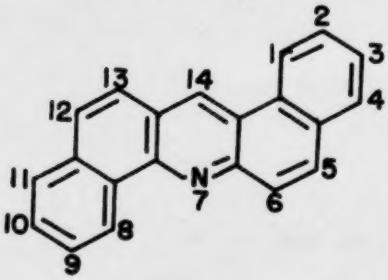
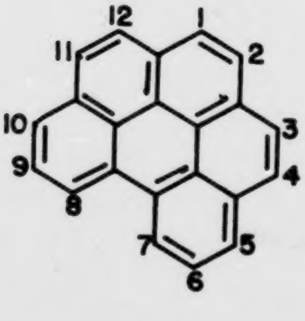
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
	Benzo[h]naphtho[1,2-f]-quinoline	279.1048	+
	Dibenz[a,j]acridine	279.1048	+
	Dibenz[c,h]acridine	279.1048	+
	Dibenz[a,h]acridine	279.1048	+
	Benzo[ghi]perylene	276.0939	+

Table A-2 (continued)

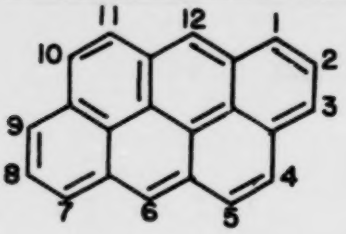
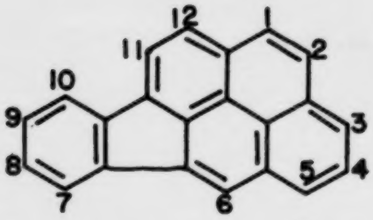
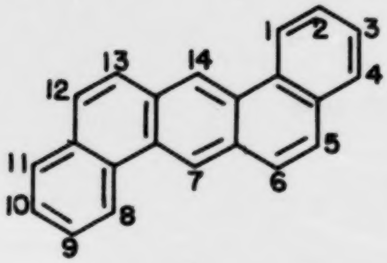
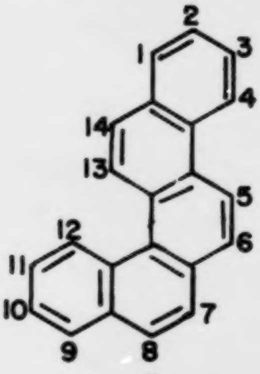
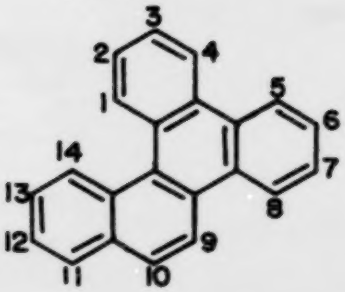
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
	Dibenzo[def,mno]-chrysene (Anthanthrene)	276.0939	0
	Indeno[1,2,3-cd]pyrene	276.0939	+
	Dibenz[a,h]anthracene	278.1096	+
	Benzo[c]chrysene	278.1096	+
	Benzo[g]chrysene	278.1096	+

Table A-2 (continued)

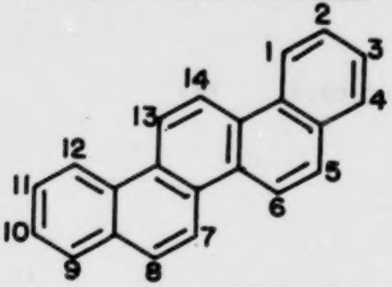
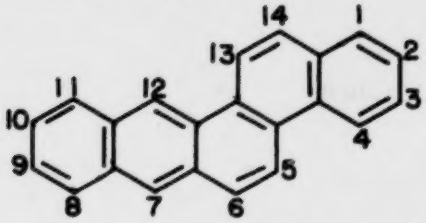
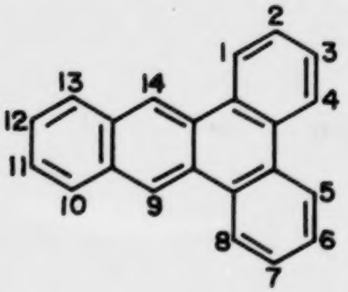
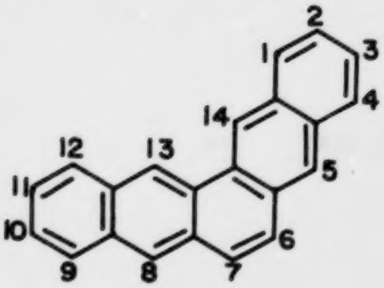
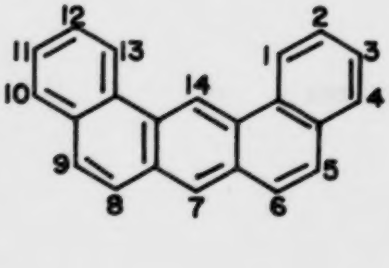
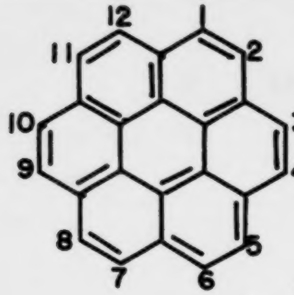
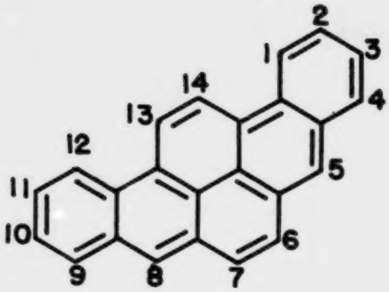
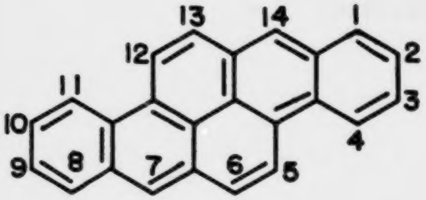
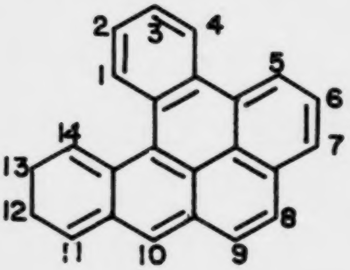
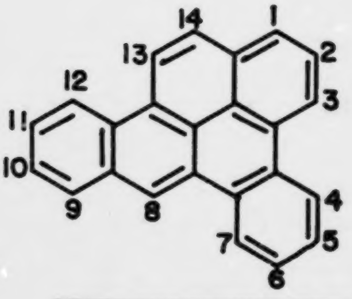
Structural Formula	Name	Molecular Weight	Carcinogenic Activity
 2 1 3 14 4 13 12 11 5 6 10 7 9 8	Picene	278.1096	0
 14 1 13 2 11 12 3 10 4 9 5 8 7 6	Benzo[b]chrysene	278.1096	0
 2 1 3 13 14 4 12 11 5 10 9 6 8 7	Benzo[b]triphenylene (Dibenz[a,c]anthracene)	278.1096	+
 2 1 3 4 14 12 13 5 11 10 6 9 8 7	Pentaphene (Dibenzo[b,h]phenanthrene)	278.1096	0
 12 2 11 13 1 3 14 10 4 9 5 8 7 6	Dibenz[a,j]anthracene	278.1096	+

Table A-2 (continued)

Structural Formula	Name	Molecular Weight	Carcinogenic Activity
 12 1 11 2 10 3 9 4 8 5 7 6	Coronene	300.0939	0/+
 2 1 3 14 4 13 12 5 11 10 6 9 8 7	Benzo[rst]pentaphene (Dibenzo[a,i]pyrene)	302.1096	++
 13 14 1 12 2 11 10 3 4 9 8 7 6 5	Dibenzo[b,def]chrysene (Dibenzo[a,h]pyrene)	302.1096	++
 2 3 4 5 1 6 14 7 13 12 8 11 10 9	Dibenzo[def,p]chrysene (Dibenzo[a,l]pyrene)	302.1096	++
 14 1 13 2 12 3 11 10 9 8 4 7 5 6	Naphtho[1,2,3,4-def]-chrysene (Dibenzo[a,e]pyrene	302.1096	++

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[a] NA = not available.

TABLE A-3

Nitroarenes Detected in Diesel-Exhaust Particulate Extracts: Molecular Formulas and Molecular Weights

Structure No.	Name	Molecular Formula	Molecular Weight
Mononitroarenes:			
1	Nitroindene	$C_9H_7NO_2$	161.16
2	Nitroacenaphthylene	$C_{12}H_7NO_2$	197.19
3	Nitroacenaphthene	$C_{12}H_9NO_2$	199.21
4	Nitrobiphenyl	$C_{12}H_9NO_2$	199.21
5	Nitrofluorene	$C_{13}H_9NO_2$	211.22
6	Nitromethylacenaphthylene	$C_{13}H_9NO_2$	211.22
7	Nitromethylacenaphthene	$C_{13}H_{11}NO_2$	213.24
8	Nitromethylbiphenyl	$C_{13}H_{11}NO_2$	213.24
9	Nitroanthracene	$C_{14}H_9NO_2$	223.23
10	Nitrophenanthrene	$C_{14}H_9NO_2$	223.23
11	Nitromethylflourene	$C_{14}H_{11}NO_2$	225.25
12	Nitromethylanthracene	$C_{15}H_{11}NO_2$	237.26
13	Nitromethylphenanthrene	$C_{15}H_{11}NO_2$	237.26
14	Nitrotrimethylnaphthylene	$C_{13}H_{13}NO_2$	215.25
15	Nitrofluoranthene	$C_{16}H_9NO_2$	247.25
16	Nitropyrene	$C_{16}H_9NO_2$	247.25
17	Nitro(C_2-alkyl)anthracene	$C_{16}H_{13}NO_2$	251.29
18	Nitro(C_2-alkyl)phenanthrene	$C_{16}H_{13}NO_2$	251.29
19	Nitrobenzofluorene	$C_{17}H_{11}NO_2$	261.28
20	Nitromethylfluoranthrene	$C_{17}H_{11}NO_2$	261.28
21	Nitromethylpyrene	$C_{17}H_{12}NO_2$	262.29
22	Nitro(C_3-alkyl)anthracene	$C_{17}H_{15}NO_2$	265.31
23	Nitro(C_3-alkyl)phenanthrene	$C_{17}H_{15}NO_2$	265.31
24	Nitrochrysene	$C_{18}H_{11}NO_2$	273.29
25	Nitrobenzanthracene	$C_{18}H_{11}NO_2$	273.29
26	Nitronaphthacene	$C_{18}H_{11}NO_2$	273.29
27	Nitrotriphenylene	$C_{18}H_{11}NO_2$	273.29
28	Nitromethylchrysene	$C_{19}H_{13}NO_2$	287.32
29	Nitromethylbenzanthracene	$C_{19}H_{13}NO_2$	287.32
30	Nitromethyltriphenylene	$C_{19}H_{13}NO_2$	287.32
31	Nitrobenzopyrene	$C_{20}H_{11}NO_2$	297.31
32	Nitroperylene	$C_{20}H_{11}NO_2$	297.31
33	Nitrobenzofluoranthene	$C_{20}H_{11}NO_2$	297.31
Polynitroarenes:			
34	Dinitromethylnaphthylene	$C_{11}H_9N_2O_4$	233.20
35	Dinitrofluorene	$C_{13}H_8N_2O_4$	256.22
36	Dinitromethylbiphenyl	$C_{13}H_{10}N_2O_4$	258.23
37	Dinitrophenanthrene	$C_{14}H_8N_2O_4$	268.23
38	Dinitropyrene	$C_{16}H_8N_2O_4$	292.25
39	Trinitropyrene	$C_{16}H_7N_3O_6$	337.25
40	Trinitro(C_5-alkyl)fluorene	$C_{18}H_{17}N_3O_6$	371.35
41	Dinitro(C_6-alkyl)fluorene	$C_{19}H_{19}N_2O_4$	339.37
42.	Dinitro(C_4-alkyl)pyrene	$C_{20}H_{16}N_2O_4$	348.36

Table A-3 (continued)

Structure No.[a]	Name	Molecular Formula	Molecular Weight
Nitro-oxyarenes:			
43	Nitronaphthaquinone	$C_{10}H_5NO_4$	203.15
44	Nitrodihydroxynaphthalene	$C_{10}H_8NO_4$	206.18
45	Nitronaphthalic acid	$C_{10}H_8NO_4$	206.18
46	Nitrofluorenone	$C_{13}H_7NO_3$	225.20
47	Nitroanthrone	$C_{14}H_9NO_3$	239.23
48	Nitrophenanthrone	$C_{14}H_9NO_3$	239.23
49	Nitroanthraquinone	$C_{14}H_7NO_4$	253.21
50	Nitrohydroxymethylfluorene	$C_{14}H_{11}NO_3$	241.25
51	Nitrofluoranthone	$C_{16}H_8NO_3$	262.24
52	Nitrofluoranthenequinone	$C_{16}H_7NO_4$	277.24
53	Nitropyrenequinone	$C_{16}H_8NO_4$	278.24
54	Nitropyrone	$C_{16}H_9NO_3$	263.25
55	Nitrodimethylanthracene carboxaldehyde	$C_{17}H_{12}NO_3$	278.29
56	Nitrodimethylphenanthrene carboxaldehyde	$C_{17}H_{12}NO_3$	278.29
Other nitrogen compounds:			
57	Benzocinnoline	$C_{12}H_8N_2$	180.21
58	Methylbenzocinnoline	$C_{13}H_{10}N_2$	194.24
59	Phenylnaphthylamine	$C_{16}H_{13}N$	219.29
60	(C_2-Alkyl)phenylnaphthylamine	$C_{18}H_{17}N$	247.34

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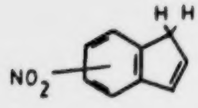
[a] Structure numbers refer to structures in Table A-4.

TABLE A-4

Structures of Nitroarenes[a]



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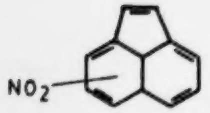

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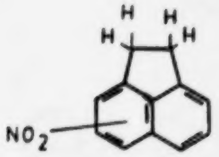

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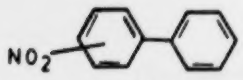

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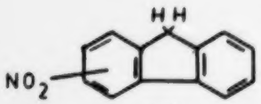

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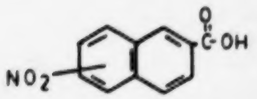

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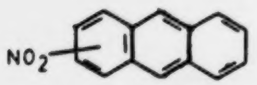

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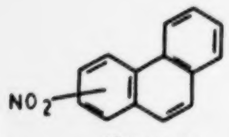

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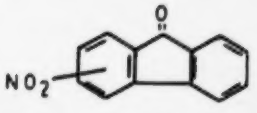

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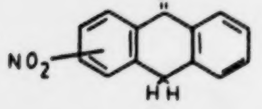

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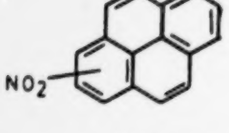

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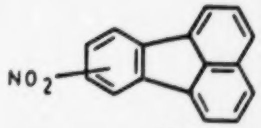

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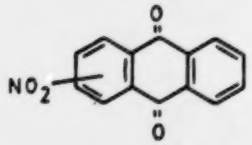

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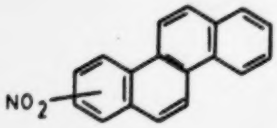

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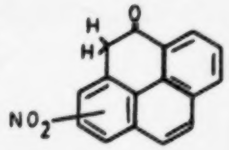

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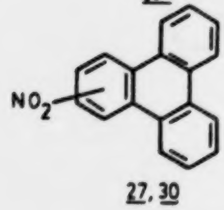

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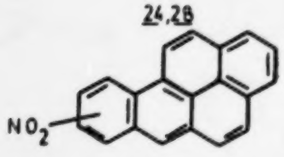

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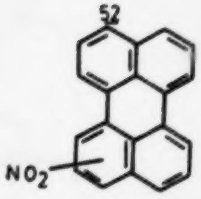

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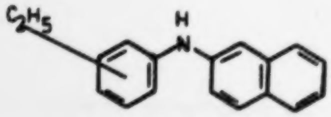

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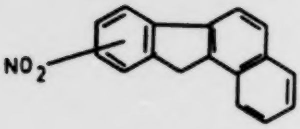

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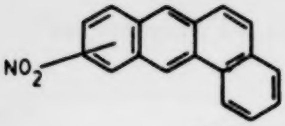

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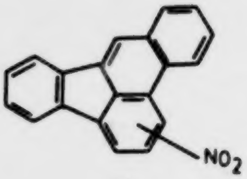

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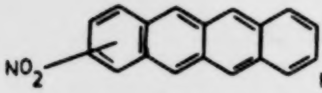

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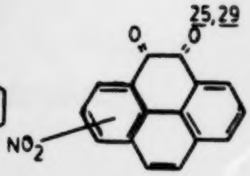

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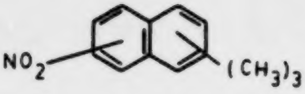

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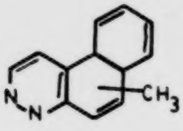

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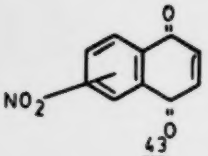

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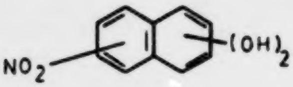

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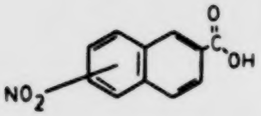

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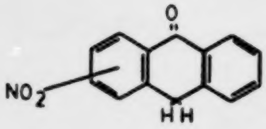

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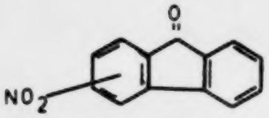

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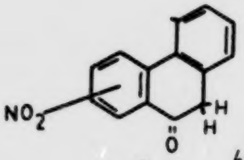

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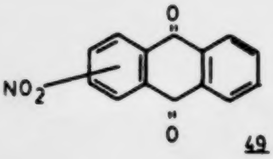

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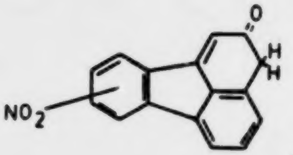

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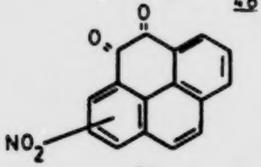

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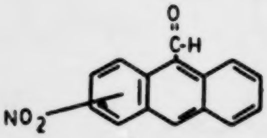

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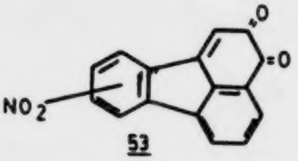

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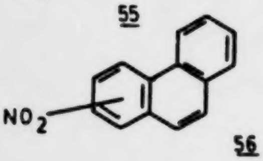

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[a] Numbers under structures refer to compounds listed in Table A-3.

APPENDIX B

POLYCYCLIC AROMATIC HYDROCARBONS IN THE AMBIENT ATMOSPHERE

Compound	Ambient concentration, ng/m^3	References
Unsubstituted:		
Biphenyl	a	1
Naphthalene	0.05-0.35	6
Anthracene	0.07-6.15	6
Phenanthrene	0.04-25	6
Benz[a]anthracene	0.5-22	6
Dibenz[ac]anthracene	0.03-4.5	6
Benzo[c]phenanthrene	0.04-1.0	6
Benzo[a]fluorene	0.8	6
Benzo[b]fluorene	0.1-1.1	6
Dihydrobenzo[a,b, and c]fluorenes	0.03-0.9	1,6
Fluoranthene	0.1-41	6
Benzo[b]fluoranthene	0.1-7.4	6
Benzo[j]fluoranthene	0.2-4.4	6
Benzo[k]fluoranthene	0.14-20	6
Benzo[ghi]fluoranthene	0.9-9.1	6
Pyrene	0.1-35	6
Benzo[a]pyrene	0.1-75	6
Benzo[e]pyrene	0.1-42	6
Anthanthrene (dibenzo[cdjk]pyrene)	0.1-6	6
Dibenzopyrenes (4 isomers)	a,b	4,6
Indeno(1,2,3-cd)pyrene	1-12.8	6
Chrysene	0.2-39	6
Perylene	0.1-5	6
Benzo[ghi]perylene	0.2-46	6
Coronene	0.2-48	6
Picene	a	1
Benzo[c]phenanthrene	a	1
Benzo[b]chrysene	a	1
Benzo[c]tetraphene	a	1
Hexahydrochrysene	a	1
Dihydrobenzo[c]phenanthrene	a	1
Dihydrobenz[a]anthracene	a	1
Dihydrochrysene	a	1
Benzacenaphthylene	b	4
Binaphthyl (3 isomers)	b	4
Quarterphenyl	b	4
Diphenylacenaphthalene	b	4

Compound	Ambient concentration, ng/m^3	References
Alkyl-substituted:		
Methylanthracene	0.22-0.66	6
1-, 2-, 3-, and 9-Methylphenanthrenes	b	4
1-Methylpyrene	0.01-0.15	6
1-, 2-, and 4-Methylpyrenes	b	4
Ethylanthracene[c]	a	1,4
Ethylphenanthrene[c]	1,4	
Methylfluoranthene (5 isomers)	a	1,4
Methylbenz[a]anthracene[d]	a	1
Methylchrysene[d]	a	1
Methylbenzo[bk]fluoranthene	a	1
Methylbenzo[ae]pyrene	a	1
Methylbenzopyrenes or benzofluoranthenes (5 isomers)	b	4
4H-Cyclopenta[def]phenanthrene	b	4
Methyl 4H-cyclopenta[def]phenanthrene	b	4
Ethyl 4H-cyclopenta[def]phenanthrene (5 isomers)	b	4
Ethylmethyl 4H-cyclopenta[def]-phenanthrene	b	4
Ethylmethyl anthracene or phenanthrene	b	4
Ethylpyrene or fluoranthene (4 isomers)	b	4
Ethylmethylpyrene or fluoranthene (3 isomers)	b	4
Methylbenzo[c]phenanthrene	b	4
Methylbenzo[ghi]fluoranthene	b	4
Ethylchrysene or benz[a]anthracene (7 isomers)	b	4
Methylbinaphthyl (4 isomers)	b	4
Methyldibenzanthracene	b	4
N-Hetero (aza):		
Acridine	0.04	6
Methylacridine	0.007	6
Benz[a]acridine	0.2	6
Benz[c]acridine	0.1-1.5	6
Dibenz[aj]acridine	0.04	6
Dibenz[ah]acridine	0.08-0.1	6
Carbazole	1.9	6
Quinoline	0.02-0.6	6
Methylquinoline	0.03	6
2,6-Dimethylquinoline	0.03	6
Dimethylquinolines	0.04-0.09	6
Ethylquinolines	0.01-0.02	6
C_3 Alkylquinolines	0.01	6

Compound	Ambient concentration, ng/m^3	References
Benzo[f]quinoline	0.01-0.2	6
Benzo[h]quinoline	0.01-0.3	6
11-Indeno[1,2b]quinoline	0.1	6
Phenanthridine	0.02	6
Isoquinoline	0.14-0.18	6
Methylisoquinolines	0.17-0.31	6
Dimethylisoquinolines	0.06	6
Ethylisoquinolines	0.07-0.16	6
C_3 Alkylisoquinolines	0.03	6
Benz[f]isoquinolines	0.03-0.11	6
4-Azafluorene	0.005	6
4-Azapyrene and isomers	0.02-13	6
1-Azafluoranthene	trace-3	6
Benzo[c]cinnoline	1.0	6
2-Methylindole	2.0	6
Benzo[a]carbazole	a	1
Benzo[c]carbazole	a	1
Phenoxazine	a	1
C_4 Alkylquinolines	a	1
Methylphenanthridines	a	1
Methylbenzoquinolines	a	1
Methylbenzoisoquinolines	a	1
Azabenzofluorenes	a	1
Methylazapyrenes	a	1
Methylazafluoranthenes	a	1
Azabenz[a]anthracene	a	1
Azachrysenes	a	1
Azabenzopyrenes	a	1
Azabenzofluoranthenes	a	1
Dibenzoquinolines	a	1
Dibenzoisoquinolines	a	1
Quinones:		
9,10-Anthraquinone	b	5
Benzo[a]pyrene 6,12-quinone	b	5
Benzo[a]pyrene 1,6-quinone	b	5
Benzo[a]pyrene 3,6-quinone	b	5
Dibenzo[b,def]chrysene 7,14-quinone	b	5
Phenalen-1-one	0.3-17	7
Benzanthrone	0.6-48	5,6
Perinaphthanone	a	6
Carboxylic acids:		
Naphthalene carboxylic acid	a	1
Phenanthrene carboxylic acid	a	1
Anthracene carboxylic acid	a	1
Pyrene carboxylic acid	a	1

Compound	Ambient concentration, ng/m^3	References
Phenols:		
Hydroxyanthracene	a	1
Hydroxyphenanthrene	a	1
Hydroxypyrene	a	1
Hydroxyfluoranthene	a	1
S-Hetero:		
Benzothiazole	0.014-0.02	2
Dibenzothiophene	b	4
Methyldibenzothiophenes (3 isomers)	b	4
Ethyldibenzothiophene	b	4
Benzo[def]dibenzothiophene	b	4
Naphthobenzothiophenes (3 isomers)	b	4
Methylnaphthobenzothiophenes (3 isomers)	b	4
Nitro derivatives:		
1-Nitropyrene	b	7
3-Nitrofluoranthene	b	7
5-Nitroacenaphthene	b	7
6-Nitrobenzo[a]pyrene	b	3

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[a] Concentration reported in micrograms per gram of particulate matter or micrograms per gram of benzene-soluble fraction, but not in nanograms per cubic meter.

[b] Compound identified, but no concentration reported.

[c] Eight isomers of ethylanthracene/ethylphenanthrene identified.

[d] Five isomers of methylbenz[a]anthracene/methylchrysene identified.

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2. Dong, M. W., D. C. Locke, and D. Hoffmann. Characterization of aza-arenes in basic organic portion of suspended particulate matter. Environ. Sci. Technol. 11:612-618, 1977.
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APPENDIX C

HUMAN-CANCER RISK ASSESSMENT*

Malcolm C. Pike

Epidemiologic studies, animal carcinogenesis experiments, and in vitro mutagenesis and transformation assays all provide data relevant to the assessment of the human-cancer risk from exposure to PAHs.

The data used in this assessment are in the main taken from epidemiologic studies, because they refer directly to man. It is recognized that an alternative approach would have been the extrapolation of experimental animal data to humans, but the epidemiologic approach offers two advantages: the avoidance of interspecies extrapolation and the derivation of results from exposures not too different from that suffered by the general population. Epidemiologic studies often suffer from various inadequacies, such as imprecise dose measurements and poor measurement of confounding factors, and exposure is invariably to a complex mixture of PAHs and other chemicals. Extrapolation to other complex mixtures therefore inevitably involves making assumptions, and evidence from in vitro and in vivo experiments must be sought to provide a rational basis for these assumptions.

At present, the two sources of human exposure to PAHs on which data appear reliable are work around coke ovens and cigarette-smoking. The major known human cancer associated with exposure to chemical mixtures containing PAHs is undoubtedly lung cancer. Although cigarette-smoking is of overwhelming importance as a cause of lung cancer[9,40] and cigarette smoke does contain PAHs, this appendix is concerned with cigarette-smoking only insofar as the information derived from epidemiologic study of the smoking population is essential in measuring the health effects that might be expected when humans are exposed to other PAH-containing mixtures.

The quantitative relationship between cigarette-smoking and lung cancer has been thoroughly explored in many epidemiologic studies[40] and is well understood.[8,29] However, it is still far from established that the PAH content of cigarette smoke is responsible for the development of lung cancer. Epidemiologic data (mainly occupational) on the relationship between exposure to other PAH-containing mixtures and lung cancer are much less precise. The lung-cancer risks (as well as the risks of cancer at other sites) associated with such exposures have, in fact, always been measured in relation to lung-cancer rates in the "nonexposed," and cigarette-smoking has been responsible for some 90% of the lung cancers in these "nonexposed."[9] To measure the risk, rather than the relative risk, associated with these other exposures, it is essential to understand the lung-cancer risk associated with cigarette-smoking.

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*Quantitative risk assessment is a developing, rather than a precise, science. The numerical estimates in this appendix are based on a series of assumptions. The use of different assumptions or extrapolations from animal data could lead to very different conclusions. The calculated risk values at ambient concentrations are not meant to be absolute indicators of risk, but rather to indicate the region between the upper bounds of risk and the lower bound of zero risk.

DEFINITIONS

The incidence rate of a disease is the number of cases of the disease that are diagnosed during a specified period per specified unit of population.[22] The mortality rate of a disease is the number of deaths from the disease during a specified period per specified unit of population. In epidemiologic studies, the unit of time is usually a year and the unit of population is usually 100,000. All incidence (and mortality) rates quoted here for man use a period of a year, but the unit of population is 1, unless otherwise stated. If we write the incidence rate without qualification (e.g., I), it is assumed to refer to the standard condition of 1 yr and 1 person. The incidence rate is often affected by many factors, particularly age, and, if the incidence rate is for some particular subgroup, this is stated in referring to the incidence rate, and the symbol, I, for incidence rate is qualified in some way, e.g., I(t) for the incidence rate for a person of age t.

For cancers associated with a substantial cure rate or a long time between diagnosis and death, the incidence and mortality rates may be very different. For lung cancer--the major cancer discussed in this chapter--the distinction is not so important, because some 75% of newly diagnosed lung-cancer patients are dead within a year and some 90% within 3 yr.

The lifetime risk of a disease is the probability of being diagnosed as having the disease by age 70 (a "lifetime") in the absence of other causes of death. This measure has been found particularly useful in comparing human data and experimental-animal data[27] and forms the basis of current methods of extrapolating animal data to man.[34] The lifetime risk is virtually identical with the cumulative incidence rate (to age 70) used by the International Agency for Research on Cancer.[3,39]

CIGARETTE-SMOKING AS A SOURCE OF PAH EXPOSURE

Much of what has been learned about the quantitative relationship between cigarette-smoking and lung cancer over the last 30 yr may be summarized by the statement, "The excess lung-cancer incidence of a smoker, compared with a nonsmoker, is proportional to the number of cigarettes smoked per day and to the duration of smoking raised to the power 4.5."[8,40] If we write the excess incidence--or single-cause incidence[24]--of a smoker aged t years who started smoking at age w years and who smokes c cigarettes per day as $I_c(t,w)$, that statement may be expressed mathematically as

$$I_c(t,w) = ac(t - w)^{4.5}, \tag{1}$$

where the constant a is approximately 1.0×10^{-11} for U.K. smokers.[8] For U.S. smokers, the constant a must be decreased by 25-50%.[12,13,19,33] The reasons for this include the use of different tobaccos in the two countries and the mode of cigarette-smoking--in particular, British smokers tend to smoke their cigarettes down to a considerably shorter butt.[7,43] Similar reasons probably explain the existence of a range.

We may express the lung-cancer risks from cigarettes in the usual risk-assessment terms[27] of "lifetime risk" by using Equation 1. "Lifetime" is taken as 70 yr, and exposure is taken as starting at birth. If exposure is to c cigarettes per day, Equation 1 shows that the lung-cancer rate at age t will be

$$I_c(t,0) = act^{4.5}. \tag{2}$$

The lifetime risk (cumulative incidence) can be shown to be

$$CI_c(T) = 1 - \exp[-ac(70^{5.5}/5.5)]. \tag{3}$$

The lifetime lung-cancer risk associated with one U.K. cigarette per day is 2,524 per 100,000, or 2.52%.

The lung-cancer risks associated with smoking depend strongly on age at which one started to smoke, i.e., on duration of exposure (see Figure C-1). The increase in lung-cancer incidence rate of a smoker at age 60 who started to smoke at age 20 is proportional to $40^{4.5}$; if he had started at age 15, the extra rate would be proportional to $45^{4.5}$. Starting to smoke 5 yr earlier has thus increased the extra lung-cancer rate by 70% $[(45/40)^{4.5}]$, or roughly 14% for each year. To make valid comparisons between groups of persons exposed to different concentrations of PAH-containing mixtures (e.g., different occupational groups), we must therefore know their comparative smoking habits, not only in terms of number of cigarettes smoked per day, but also in terms of age at starting to smoke.

For a smoker of c cigarettes/d starting at age w and stopping at age s, the extra lung-cancer incidence rate at age t is

$$I_{c,s}(t,w) = ac(s - w)^{4.5}. \tag{4}$$

Equation 4 states that the lung-cancer incidence rate associated with cigarette-smoking remains constant at the value it had reached when smoking stopped.[5,29,40] If a person aged 60 who has smoked 30 cigarettes/d from age 20 to 40 (30 pack-yr in total) is compared with a person at the same age (60) who has smoked 15 cigarettes/d from age 20 to 60 (also 30 pack-yr in total), calculations using Equation 4 show that the latter person will have more than 11 times the lung-cancer incidence rate of the former. Thus, to understand quantitatively the effect of exposure to a PAH-containing mixture, one must know not only the total cumulative exposure, but also the time during which it is accumulated.

Hoffmann et al.[17] pointed out that the major carcinogenic activity of cigarette smoke resides in the particulate phase (the tar) and that there is good experimental evidence that cigarettes with lower tar yields are less tumorigenic to both hamster larynx and mouse skin. Lower-tar cigarettes have also been shown to be less tumorigenic to man in all epidemiologic studies that have investigated this question. Case-control studies have found that people who smoke filter-tip cigarettes (in effect, lower-tar cigarettes) have lower lung-cancer incidence rates than smokers of plain cigarettes at the same frequency,[1,44,45] and Hammond et al.[14] found, in the American Cancer Society (ACS) cohort study, that persons smoking low-tar cigarettes had lower risk of lung cancer than smokers of high-tar cigarettes (matched for numbers of cigarettes smoked per day).

Table C-1 shows the results of the ACS study: the lung-cancer mortality ratios are clearly not decreased in men in proportion to tar content, but they are nearly so in women. The latter finding suggests that the added lung-cancer risk is close to being simply proportional to tar content and that the failure to find a proportional reduction in men arises from the male smokers' having switched from high-tar to low-tar cigarettes. As Hammond et al.[14] stated: "Cigarettes with reduced tar and nicotine were not introduced until the mid 1950's. . . . Almost all of the male cigarette smokers and the great majority of the female cigarette smokers in our study began smoking cigarettes long before that date. Therefore the subjects classified here as low [tar] cigarette smokers were, with few exceptions, persons who smoked high [tar] or medium [tar] cigarettes for many years and then switched to low [tar] cigarettes." These results substantiate the linear dose-response assumption of Equation 1.

EXPOSURES TO OTHER SOURCES OF PAH-CONTAINING MIXTURES

Large-scale studies of benzo[a]pyrene in the air of the United States were conducted between 1958 and 1959 by Sawicki et al.[36] The range of BaP concentrations in urban air was from less than 1 to around 60 ng/m^3 and the median was roughly 6 ng/m^3. In contrast, BaP concentrations in nonurban air were almost always less than 1 ng/m^3, with a median of 0.4 ng/m^3. BaP concentrations have since decreased; by 1969, the median BaP concentration in urban air was less than 2 ng/m^3.[26] However, some cities were still experiencing average annual BaP concentrations of nearly 10 ng/m^3.

BaP is not a perfect indicator of either PAH in the air or its carcinogenicity,[35] and it accounts for a much smaller fraction of the carcinogenicity of cigarettes than of air.[11,42] It should be emphasized that BaP is not a good surrogate for PAHs in mixtures from different sources, although more information is available on its effects than those of other PAHs. However, a person who lives where the air contains BaP at 10 ng/m^3 and who breathes 15 m^3 of air per day would breathe in roughly the same amount of BaP as he would from smoking five old-style cigarettes (as discussed by Hoffmann et al.[17]). It is therefore not unreasonable to assume that this degree of pollution, which was very common only 20 yr ago, may cause a significant amount of lung cancer.

Studying the problem directly proves difficult, because one must be especially careful to ensure that an observed effect is not attributable to differences in smoking habits between high- and low-pollution areas. The lung-cancer incidence is affected not only by the number of cigarettes smoked, but by the tar content of the cigarettes, by how far down the cigarette is smoked, and by smokers' ages at starting to smoke and at stopping (if ever); all these aspects of smoking habits have to be considered. It is impossible to allow for all these factors accurately, so extrapolating from an extreme situation, in which small smoking-habit differences can be ignored, is likely to be the best method of estimating general air-pollution effects. Men employed in some occupations are exposed intermittently to BaP in air at up to 16,000 ng/m^3,[18] and they provide an opportunity to study lung-cancer effects in an extreme situation.

OCCUPATIONAL EXPOSURE

Many epidemiologic studies of lung cancer have involved occupational exposure to PAH-containing mixtures.[26,34] They showed that exposure to high concentrations of PAH-containing mixtures increases the risk of lung cancer.

Assuming that the exposed and nonexposed workers have the same smoking habits and that their observed lung-cancer incidence rates are r_e and r_n, respectively, we can express the lung-cancer burden from the exposure either as a ratio, $R = r_e/r_n$, or as a difference, $D = r_e - r_n$. For general risk-assessment purposes, we can express these on the basis of per-unit exposure by dividing R or D by the "exposure dose."

Both R and D are valid measures of the risk to the occupational group as a group, but they implicitly make very different assumptions about the risks to individual members of the group with different smoking habits. The relative-risk index, R, implicitly assumes that the risk of lung cancer is increased in proportion to the individual's "underlying" risk--a nonsmoker's risk is multiplied by R, and a 2-packs/d smoker's risk is also multiplied by R. The additional risk of the 2-packs/d smoker is thus an order of magnitude greater than the additional risk of the nonsmoker and double the risk of a 1-pack/d smoker. This multiplicative (sometimes referred to as synergistic) phenomenon appears to hold for lung cancer caused "jointly" by asbestos exposure and cigarette-smoking.[23]

The additional-risk index, D, implicitly assumes that the amount of increased risk of lung cancer is independent of other lung-cancer risk--a nonsmoker's risk is increased by the same absolute amount as a 2-packs/d smoker's risk.

None of the occupational studies of exposure to PAH-containing mixtures and lung cancer was conducted in such a way as to provide data to help in distinguishing between the possible models (i.e., multiplicative, additive, or something intermediate). Studies comparing urban and rural lung-cancer rates (or rates in "heavily polluted" and "lightly polluted" areas) in persons with different smoking habits do provide relevant data, but the studies generally have few deaths and do not clearly identify the correct model. Data from the study of Stocks[38] (Table C-2) and from the study of Hitosugi[16] (Table C-3) illustrate the point. In both studies, the data from the smokers are in good agreement with an additive model for the effect of "air pollution," and these data provide no evidence for a multiplicative model. The data from nonsmokers, however, confuse the picture. In Stocks's study, the effect of air pollution is smaller in the nonsmokers; in Hitosugi's study, there is no effect in nonsmokers. The problem may simply result from basing the rates on such small numbers of deaths in nonsmokers or from misclassifying the smoking habits of a few persons who died of lung cancer. Because the additive model provides such a good fit to the data on smokers, we have assumed this model in our discussion of lung-cancer risk from occupational exposure to PAH-containing mixtures.

To use occupational studies for risk-assessment purposes, we must assume that, as far as lung cancer is concerned, occupational exposures can be expressed as cigarette equivalents, i.e., that the form of Equations 1 through 4 will hold for the excess lung cancer from such exposure. We saw when discussing lung cancer and cigarette-smoking that dose and duration of exposure are critical in determining lung-cancer risk. The occupational studies must, at a minimum, provide a quantitative estimate of the dose and duration of exposure to PAH-containing mixtures. With this information and comparative information on the smoking habits of the exposed and nonexposed workers, we can estimate the absolute risk from such exposure. Unfortunately, only one occupational study with high exposure to a PAH-containing mixture supplied even this minimal information.[10]

UNITED KINGDOM GASWORKERS

In a prospective study, Doll *et al.*[6,10] followed a cohort of carbonization workers in British gasworks for up to 12 yr. Carbonization workers were exposed to BaP at an estimated average air concentration of 3,000 ng/m^3 during an 8-h shift[20] and experienced a 142% increase in lung-cancer mortality, compared with their nonexposed workmates (Table C-4). Although the smoking habits of only some 10% of the cohort were ascertained by Doll and his colleagues,[6] the exposed and nonexposed workers appear to have had very similar smoking habits, with an average current consumption of approximately 10 cigarettes/d. It is reasonable, therefore, to assign the excess lung cancer in the exposed group to their working conditions, specifically to the air to which they were exposed.

The current age of a smoker and his age at starting to smoke are both important in determining his risk of lung cancer. Likewise, both current age and age at starting as a carbonization worker are important in determining such a worker's lung-cancer risk. From the papers of Doll et al.,[6,10] one may estimate the average age of the workers at the middle year of the study to be approximately 58 yr and the average length of time exposed to be approximately 23 yr. However, this does not necessarily imply that their average age at starting such employment was 35 (58 - 23), because "the men regularly change from one type of work to another."[10]

If the men started working at age 20, their average worktime BaP exposure would be

$$3{,}000[23/(58 - 20)] = 1{,}816 \text{ ng/m}^3.$$

To express this in constant-exposure terms, we may proceed as follows:

Total BaP-carbonization
breathed per year = (1,816)(9.6)(5)(49) ng
= 4.27 mg.

That is, 9.6 = m^3 of air breathed at work in a working day--8 h at 20 L/min; 5 = working days in a week; and 49 = working weeks in a year. The total air breathed in a year is

$$(15.91)(7)(49) + (12.48)(7)(3) = 5{,}719 \text{ m}^3.$$

That is, 15.91 = [(17.28)(5) + (12.48)(2)]/7 = average m^3 breathed per day during a working week; 12.48 = m^3 breathed per day during a nonworking day; 17.28 = m^3 breathed per day during a working day--all these values calculated with assumptions of 20 L/min at work for 8 h, 6 L/min asleep for 8 h, and 10 L/min otherwise. If the gasworkers' exposure is expressed in constant-exposure terms, as though the men breathed such air throughout the day every day, the average BaP-carbonization pollution to which they were exposed is

$$4.27 \text{ mg}/5{,}719 \text{ m}^3 = 747 \text{ ng/m}^3.$$

This led to a 142% increase in the rate of lung cancer over "background," an estimate roughly 90% of which was caused by the men's smoking habits.[8]

If we assume that the relation between duration of exposure and lung-cancer risk is the same for gasworks exposure as it is for cigarette-smoking and that the men started work and started to smoke regularly at roughly the same age, we may write (in lung-cancer terms)

10 U.K. cigarettes/d = 0.9

and BaP-carbonization at 747 ng/m^3 = 1.42.

Those two equations permit us to express BaP-carbonization in terms of U.K. cigarettes as

BaP-carbonization at 47.3 ng/m^3 = 1 U.K. cigarette.

Calculation of the effect of other ages at starting carbonization-work exposure requires more elaborate computation, and the above estimate appears to be the best that can be made with the limited data available.[30]

Note that reasonable changes in the estimate of the proportion of the background lung-cancer rate that was caused by cigarette-smoking have only minor effects on this estimated equivalence. For example, if a figure of 80%, rather than 90%, is assumed, the equivalence is BaP-carbonization at 42.1 ng/m^3 = 1 U.K. cigarette.

We assumed in the above calculations that the gasworker breathed 9.6 m^3 [(8)(60)(20) L/min] of air at work each working day. The "average" adult breathes roughly half this amount at work. If we assume further that gasworkers and the average man breathe similarly at other times, then the average man breathes 4,543 m^3 of air per year, or 79% (4,543/5,719) as much air as a gasworker. The above equivalent of 47.3 must therefore be divided by this figure to make the exposure applicable to "average" man. Our best estimate is thus finally 59.5, i.e., BaP-carbonization at 59.5 ng/m^3 = 1 U.K. cigarette. We estimate from Equation 3 that the lifetime lung-cancer risk associated with exposure to BaP-carbonization at 1 ng/m^3 would be 43/100,000.

UNITED STATES COKE WORKERS

Lloyd and his colleagues[21,32] found in cohort studies of U.S. steelworkers that coke-oven workers experienced a substantial excess risk of lung cancer. These workers, like the British gasworkers, are exposed to the products of coal carbonization. Compared with nonoven workers at the same plants, the coke-oven workers as a group had 2.8 times the lung-cancer mortality rate; and coke-oven workers who had more than 5 yr of "topside" exposure had 6.9 times the lung-cancer mortality rate. No data were given on the smoking habits of these workers or of nonexposed workers, on length of employment, on age, or on average BaP exposure. However, Jackson *et al.*[18] found average BaP concentrations on the battery roof of a coke-manufacturing plant of 6,700 ng/m^3. If this is taken as the BaP exposure of the topside workers, these estimates of lung-cancer risk are remarkably compatible with those from the study of British carbonization workers.

The British carbonization workers had a relative risk of lung cancer of 2.42 at a BaP exposure of 3,000 ng/m^3, so we may write

Nonexposed British lung-cancer rate = 1.0,
Carbonization workers' rate = 2.42,
Increment per 1,000 ng/m^3 of
BaP-carbonization exposure = (2.42 - 1.0)/3 = 0.47.

At the time of these surveys, the age-adjusted U.S. national lung-cancer mortality rate was just half the British rate.[12,37] Taking into account this fact and the relative risk of 6.9 for the U.S. topside workers, we may write

Nonexposed U.S. lung-cancer rate = 0.5,
Topside workers' rate = 0.5 x 6.9 = 3.45,
Increment per 1,000 ng/m^3 of
BaP-carbonization exposure = (3.45 - 0.5)/6.7 = 0.44.

The experience of coke-oven workers in the U.S. steel industry is thus in very close agreement with the British data on gasworkers in BaP-exposure terms.

LONDON DIESEL-BUS GARAGE WORKERS

The lung-cancer incidence among diesel-bus garage workers employed by the London Transport Authority (LTA) has been examined for the period 1950-1974.[15,31,41] These men were exposed to more diesel emission than other LTA employees, but they showed no greater risk of lung cancer than the other employees.

No detailed information on the garage workers' duration of exposure to diesel fumes has been published, but the concentration of smoke was measured inside and outside selected garages.[2,41] Waller[41] concluded that "the indications are that the overall exposure of garage workers to benzo[a]pyrene during their working lives would not differ much from those of the general population." The BaP exposure of the U.K. gasworkers discussed above was some 100 times background and was associated with a 142% increase in lung-cancer rates. It is therefore hardly surprising that the very small increase over background pollution in a diesel garage (certainly less than a twofold increase) did not produce an epidemiologically measurable effect. Other possible biases in comparing the LTA workers in different job categories were discussed at length by Harris.[15] The study must be considered noninformative, rather than negative; we have discussed it here because it was used as an important data source in recent NRC reports[15,25] on the impact of particulate emission from diesel-powered light-duty vehicles.

OTHER OCCUPATIONALLY EXPOSED GROUPS

Results of other studies of groups of workers exposed to PAH-containing mixtures were reviewed recently.[25,34] None of these studies provided evidence of very high exposure; most provided no measure of actual length or intensity of exposure to PAH-containing mixtures or comparative cigarette-smoking habits. Their results are not useful for purposes of quantitative risk assessment.

GENERAL AIR-POLLUTION EXPOSURE

Studies of the effects of exposure to general air pollution have been reviewed in numerous reports.[4,9,26,34] These reviews have found that lung-cancer rates (as well as rates of cancer at almost all other sites) are higher in urban (i.e., "polluted") than in rural areas (Santodonato *et al.*,[34] Table 6-47). Interpretation of the increased rates is invariably confounded, however, by lack of information on the possible contribution of occupation-induced lung cancer, the possibility of greater accuracy of death certification in urban areas, and, most critically, the lack of detailed information on smoking history.

The confounding by occupationally induced lung cancer and more accurate death certification in urban areas is unlikely to be the explanation of most of the urban excess.[4] The confounding by lack of smoking-history information is likely to be the most important.[9] We have seen (Figure C-1) that the lung-cancer risk among cigarette-smokers depends strongly on age at starting to smoke, and this holds true even into old age. For valid comparison of lung-cancer rates between urban and rural areas, which allows for smoking-habit differences, it is therefore necessary to know, at a minimum, not only the current smoking habits in the areas being compared, but also the past smoking habits in these areas. In most countries, cigarette-smoking became popular much later in rural than in urban areas; this itself ensures (even allowing for current smoking habits) that lung-cancer rates will be higher in urban than in rural areas of such countries.

The above arguments make urban-rural comparisons a very weak basis for evaluating the effect of general air pollution on lung-cancer rates. Moreover, most urban-rural comparisons are of no use for quantitative risk-assessment purposes, because they include no estimate of PAH concentrations in the air in the different areas.

LIVERPOOL-NORTH WALES COMPARISON

The urban-rural comparison study undertaken by Stocks[38] covering the years 1952-1954 in Liverpool (urban) and parts of North Wales (rural) is perhaps unique, in that he not only measured air pollution, but also addressed the issue of long-term smoking habits. The air pollution in the two areas was measured in terms of average BaP concentration over a 2-yr period starting in October 1954: the average BaP concentration in the air was 6.7 ng/m^3 in the rural area and 59.2 ng/m^3 in the urban area. Stocks addressed the issue of long-term smoking habits by showing that, in men aged 50-59 at the time of the survey in 1953-1955, the urban-rural contrast in smoking habits did not differ from that of 20 yr earlier.

Table C-2 shows the calculated age-standardized lung-cancer rates by smoking category in the two areas. The difference in lung-cancer rates between the two areas, averaged over the smoking categories, is approximately 74. Assuming that this difference is due totally to general air pollution, which was mainly the result of inefficient burning of coal, we may express these rates approximately in terms of Equation 2, with t taking the value 55, and hence in terms of equivalent U.K. cigarettes. These calculations estimate the effect of the additional BaP air pollution in the urban area as the equivalent of 1.09 U.K. cigarettes. Thus, we estimate

BaP-coal-burning at 52.5 ng/m^3 = 1.09 U.K. cigarettes
or BaP-coal-burning at 48.2 ng/m^3 = 1 U.K. cigarette.

Therefore, even though Stocks failed to address the issue of lifelong smoking habits satisfactorily, his data suggest a figure for BaP-coal-burning that is not much different from BaP-carbonization. If we use only the data on nonsmokers in Table C-2 to estimate the effect of BaP-coal burning, we find that

BaP-coal-burning at 128 ng/m^3 = 1 U.K. cigarette.

RATES IN NONSMOKERS

The study of Stocks[38] has been criticized, because he obtained data on many of the lung-cancer patients from relatives after the patients' deaths. This would especially tend to exaggerate the lung-cancer rates in the "nonsmokers."[4] Doll[5] suggested that a more accurate lung-cancer figure for nonsmokers could be obtained by combining the data on lifelong nonsmokers from the prospective studies of Kahn[19] and Hammond[13] in the United States. The combined data (Table C-5) show a lung-cancer mortality rate for nonsmokers roughly 45% of that found for nonsmokers in rural North Wales by Stocks. This is the relevant comparison, because the average BaP concentration in urban air in the United States[36] in 1959 was roughly 6 ng/m^3--a figure very close to that of rural North Wales in 1954.

Doll[5] showed that Equation 2 provided an excellent fit to the combined nonsmoker data from Kahn[19] and Hammond[13] (see Table C-5), and the best fit is obtained with the equivalent number of U.K. cigarettes (smoked from birth) set at 0.14. If these lung cancers were due totally to BaP-U.S. pollution, we could conclude

BaP-U.S. pollution at 6 ng/m^3 = 0.14 U.K. cigarette
or BaP-U.S. pollution at 42 ng/m^3 = 1 U.K. cigarette.

This may be considered a reasonable upper limit of the potency of BaP-U.S. pollution in nonsmokers.

REGRESSION STUDIES

A multiple-regression analysis undertaken for the National Research Council Subcommittee on Particulate Polycyclic Organic Matter[26] attempted to "explain" the annual lung-cancer death rates (per 100,000) in 1950-1969, Y, in the 48 conterminous states of the United States by the independent variables

X_1 = cigarette sales per person over 15 yr old (1963), in dollars,

and X_2 = BaP in air, in ng/m^3 (1967-1969).

A typical result obtained was

$$Y = 89.4 + 1.44\ X_1 + 7.05\ X_2$$

for white men aged 55-64. The observed average lung-cancer mortality rate for such men for the 48 states was 140.6.

There are a number of major problems with this approach, which are discussed at length in the report--in particular, the crudity of both the cigarette-consumption data and the air-pollution figure for a whole state. The regression equations also predict lung-cancer mortality rates in the absence of smoking or air pollution that are much greater than the observed lung-cancer incidence in nonsmokers. For example, Doll[5] gave a figure of 13.9 (compared with the above figure of 89.4) for the lung-cancer mortality rate in this age group on the basis of the combined results of Kahn[19] and Hammond.[13]

Other regression studies have similar problems, leaving them useless for quantitative risk assessment.

COMPARATIVE CARCINOGENICITY OF DIFFERENT AIR-POLLUTION MIXTURES

The available epidemiologic evidence reviewed above suggests that the carcinogenic potencies of various air-pollution mixtures (coal carbonization, coal-burning, and general U.S. pollution) are similar when expressed in terms of the BaP content of the mixtures (Table C-6). We have no useful epidemiologic data on cases in which the major contributor to air pollution has been mobile sources; to estimate the effects of such air pollution, we must use the results of animal-carcinogenesis studies and short-term mutagenesis assays.

This approach was used by Harris;[15] Table C-7 shows the assay results he considered. Tables C-8 and C-9 show the relative potencies of the various contributors to air pollution computed from the data in Table C-7. Coke-oven extract is taken as the standard, and the results are expressed on a constant-weight-of-extract basis in Table C-8 and a constant-weight-of-BaP basis in Table C-9. For example, with the SENCAR mouse assay, roofing-tar extract is 0.255 (0.535/2.101) times as potent as coke-oven extract on an equal-weight basis and 0.137 [(0.255)(478/889)] times as potent as coke-oven extract on a constant-weight-of-BaP basis.

Tables C-6 and C-9 may be used together to predict the lung carcinogenicity of exposure to spark-ignition or diesel engine exhaust. Table C-9 suggests that exposure to a fixed amount of BaP from a Mustang mixture will be between 0.06 and 2.2 times as carcinogenic as such exposure to coke-oven pollution. The different vehicles tested vary widely in diesel-exhaust extract. The results shown in Table C-9 suggest that exposure to a fixed amount of BaP from diesel exhaust will be between 0.1 and 89 times as carcinogenic as such exposure to coke-oven pollution.

If we consider the L5178Y+ assay as the assay of choice, the predicted lifetime (age 70) lung-cancer risk associated with exposure to air polluted by a 1-ng/m^3 BaP source for mobile-source emission is given in Table C-10.

OTHER CANCER SITES

Increased rates of cancer at sites other than lung were observed in the study of British gasworkers[10] and in the study of U.S. coke-oven workers.[32]

In the study of British gasworkers, an excess risk was noted for cancer of the bladder (age-adjusted rate per 1,000 of 0.37 vs. 0.12 expected), for cancer of the skin and scrotum (0.10 vs. 0.00), and for cancer at all other sites combined (2.73 vs. 2.27). Because the excess risk of cancer of the skin and scrotum is extremely unlikely to be due to inhalation exposure, the maximal excess rate of all cancer except lung cancer that can be attributed to gasworks exposure is 0.71 (3.10 - 2.39). The comparable figure for lung cancer is 2.12 (3.61 - 1.49). Lung cancer therefore accounts for at least 75% (2.12/2.83) of the excess cancer associated with this British gasworks pollution.

Similar calculations from the study of Redmond *et al.*[32] for men employed 5 yr or more in the most polluted area (topside) of the U.S. coke ovens show that lung cancer accounted for at least 83% (17.6/21.1) of the excess cancer associated with U.S. coke-oven air-pollution exposure.

FOOD

The estimated daily intake of BaP in food is 160-1,600 ng (see Table 6-25). No epidemiologic studies are available to permit one to estimate the possible carcinogenic effect of such an intake of BaP, and recourse must be made to animal experiments.

The experiment of Neal and Rigdon,[28] referred to in Chapter 4, found that BaP administered to mice in their diet produced forestomach tumors. With the extrapolation procedure used by the National Research Council Safe Drinking Water Committee,[27] it can be calculated that a daily human intake of 47 ng of BaP would lead to a lifetime risk of 1 in 100,000. With this estimate, we may calculate that the daily intake of 160-1,600 ng of BaP translates into an estimated lifetime cancer risk of 3.4-34 in 100,000. The estimated daily intake of PAHs in food is 10 times the intake of BaP (see Table 6-25), so one would estimate the total lifetime cancer risk associated with exposure to BaP and other PAHs in food at something less than 10 times these figures.

TABLE C-1

Lung-Cancer Mortality Ratios for Smokers of High-, Medium-, and Low-"Tar" Cigarettes, 1960-1972[a]

"Tar" Content, mg/cigarette	Mortality Ratio	
	Males	Females
High (30)	1.0	1.0
Medium (22.5)	0.95	0.80
Low (15)	0.81	0.60

---

[a] Data from Hammond *et al.*[14]

TABLE C-2

Lung-Cancer Mortality Rates of Men in Rural (North Wales) and Urban (Liverpool) Areas, 1952-1954, by Past Smoking Habits[a]

Smoking Category	Lung-Cancer Rate[b] Rural	Urban
Nonsmokers	22 (2)	50 (3)
Cigarette-smokers:		
App. 10 cigarettes/d	68 (23)	168 (71)
App. 20 cigarettes/d	147 (36)	248 (140)
App. 35 cigarettes/d	317 (33)	344 (138)

---

[a] Data from Stocks (p. 80).[38]

[b] Per 100,000 per year, standardized for age. Figures in parentheses are numbers of lung-cancer deaths.

TABLE C-3

Lung-Cancer Mortality Rates of Men, Aged 35-74, in Japan, by Area Pollution and Smoking Habits[a]

	Lung-Cancer Rate[b]		
Smoking Category	Low Pollution	Intermediate Pollution	High Pollution
Nonsmokers	11.5 (5)	3.8 (1)	4.9 (1)
Exsmokers	26.2 (11)	42.6 (7)	61.7 (7)
Cigarette-smokers:			
1-14 cigarettes/d	10.6 (9)	14.2 (10)	23.5 (14)
15-24 cigarettes/d	14.7 (18)	19.1 (17)	27.0 (17)
25+ cigarettes/d	36.3 (19)	15.8 (4)	46.4 (9)

---

[a] Reprinted from National Research Council[26] (Table 17-26); data derived from Hitosugi.[16]

[b] Per 100,000 per year, standardized for age. Figures in parentheses are numbers of lung-cancer deaths.

TABLE C-4

Smoking Habits and Lung-Cancer Mortality Rates of British Gasworkers

Population	Non-smokers, %	Ex-smokers, %	Continuing Smokers, %					Lung-Cancer Mortality Rate[a]
					Cigarettes/d			
			Pipe	Mixed	1-9	10-19	20+	
"Exposed" gasworkers	8.3	10.2	6.7	4.4	18.1	38.5	13.9	3.61
Other gasworkers	5.8	15.3	5.9	6.2	17.8	35.5	13.4	1.49

---

[a] Per 100,000 per year, standardized for age.

TABLE C-5

Lung-Cancer Mortality in U.S. Nonsmokers[a]

Age, yr	Annual Mortality Rate, per 100,000
35-44	2.8
45-54	5.8
55-64	13.9
65-74	25.6
75-84	49.4

---

[a] Reprinted with permission from Doll,[5] based on data from Kahn[19] and Hammond.[13]

TABLE C-6

Estimates of Lifetime (70 yr) Lung-Cancer Risk from Exposure to BaP Source at 1 ng/m^3

Study Population	Lifetime Lung-Cancer Risk, per 100,000	Reference
Gasworkers	43	Doll *et al.*[6,10]
Liverpool-North Wales:		Stocks[38]
All men	53	
Nonsmokers	20	
Nonsmokers	<61	Doll[5]

TABLE C-7

Estimates of Potency of Organic Extracts from Various Sources of Air Pollution[a]

Source	BaP[b]	SENCAR Mice[c]	Viral Trans-formation[d]	L5178Y[e]	
				-	+
Coke oven (gasworks)	478	2.101	0.859	0.726	9.963
Roofing tar	889	0.535	2.066	0.311	9.556
Caterpillar 3304 D	2	0.011	0.039	0.156	0.049
Oldsmobile 350 D	2	0.156	0.067	0.270	0.764
Volkswagen Turbo D	26	--	0.128	2.545	1.012
Mustang II 302 V-8, catalyst	103	0.027	0.204	0.348	0.990

---

[a] Data from Harris.[15]

[b] Nanograms of BaP per milligram of extract.

[c] Tumor initiation in SENCAR mice, papillomas/mouse per milligram of extract at 27 wk.

[d] Enhancement of SA7 viral transformation in Syrian hamster embryo cells, transformations per 2 x 10^6 cells per nanogram of extract per milliliter.

[e] L5178Y mouse-lymphoma mutagenesis assay (average mutant colonies/10^6 survivors per microgram of extract per milliliter) without (-) and with (+) metabolic activation.

TABLE C-8

Estimates of Potency of Organic Extracts from Various Sources of Air Pollution, Relative to Potency of Coke-Oven Extract[a]

Source	BaP	SENCAR Mice	Viral Trans-formation	L5178Y	
				-	+
Coke oven (gasworks)	1	1	1	1	1.0
Roofing tar	1.86	0.255	2.41	0.428	0.959
Caterpillar 3304 D	0.00418	0.00524	0.0454	0.215	0.00492
Oldsmobile 350 D	0.00418	0.0743	0.0780	0.372	0.0767
Volkswagen Turbo D	0.0544	--	0.149	3.51	0.102
Mustang II 302 V-8, catalyst	0.215	0.0129	0.237	0.479	0.0994

---

[a] Coke-oven response and BaP content (ng/mg of extract) set at 1.0. See Table C-7.

TABLE C-9

Estimates of Potency of Organic Extracts from Various Sources of Air Pollution, in Terms of Fixed BaP Content and Relative to Potency of Coke-Oven Extract[a]

Source	SENCAR Mice	Viral Transformation	L5178Y	
			-	+
Coke oven (gasworks)	1	1	1	1
Roofing tar	0.137	1.29	0.230	0.516
Caterpillar 3304 D	1.25	10.9	51.4	1.18
Oldsmobile 350 D	17.7	18.6	88.9	18.3
Volkswagen Turbo D	--	2.74	64.4	1.87
Mustang 302 V-8, catalyst	0.0596	1.10	2.22	0.461

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[a] See Tables C-7 and C-8.

TABLE C-10

Estimated Cumulative Lung-Cancer Incidence to Age 70 Due to Lifelong Exposure to Various Pollutant Sources at BaP of 1 ng/m^3[a]

Source	Cumulative Incidence, per 100,000
Coke oven	43 (0.043%)
Caterpillar 3304 D	51 (0.051%)
Oldsmobile 350 D	787 (0.787%)
Volkswagen Turbo D	80 (0.080%)
Mustang 302 V-8, catalyst	20 (0.020%)

---

[a] Based on Table C-6 and L5178Y+ in Table C-9.

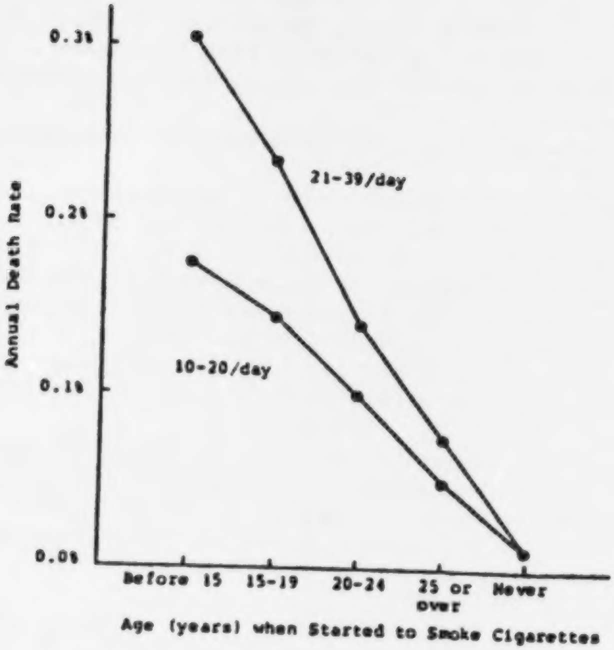

FIGURE C-1. Data on U.S. veterans.[19] Lung-cancer mortality at ages 55-64 among current smokers of cigarettes only, in relation to the age when cigarette-smoking began (although this was perhaps not when regular consumption of substantial numbers of cigarettes began). Reprinted from Doll and Peto.[9]

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APPENDIX D

PUBLIC DECISION-MAKING WITH RESPECT TO ATMOSPHERIC PAH SOURCES AND EMISSIONS

Lawrence J. White

Among the possible justifications for public decision-making with respect to PAH sources and emissions would be a finding that PAHs pose an actual or potential (and nontrivial) threat to human health. This appendix uses the cancer-risk estimates developed in Appendix C. It assumes that benzo[a]pyrene (BaP) can be used as a proxy for PAHs and that human exposure to BaP in the ambient air at an average concentration of 1 ng/m^3 over an entire lifetime has the effect of increasing by 0.02-0.06% the risk of dying prematurely (at or before the age of 70) because of lung cancer. Although the appropriateness of BaP as a surrogate for PAHs in general has been questioned, it has been so used extensively in the past, and much of the available information refers to it as an indicator for exposure to PAHs. The estimates of Appendix C are also based on this application. The focus of this appendix is on the lung-cancer consequences of human exposure to atmospheric sources of PAHs.

The rationale for public decision-making with respect to PAH emissions from atmospheric sources is explored first, followed by discussions of the general problems of developing the appropriate decision-making tools, deciding on appropriate levels of control, and choosing appropriate means of implementing the decisions. The principles developed are then applied to PAH emissions of various sources, within the constraints of the limited amount of information that is available. These efforts should be viewed primarily as illustrative and approximate, because the data available are rough and approximate. Complete analysis would require a direct linking of the damage caused by an air pollutant to the sources of its emission. For that, the following would be needed: data on emissions of the pollutant, a model of the pollutant's dispersion and possible transformation or decay during dispersion, estimates of the resulting concentrations in the ambient air, data on human exposure to those concentrations, and a model of the exposure dose-response relationship. Reliable estimates of the costs and consequences of control are also needed. With respect to all these subjects, the relevant data on PAHs are scanty and approximate, and compromises will have to be made. Some estimates may be in error by as much as an order of magnitude. Nevertheless, the results should be informative and point the way toward further appropriate study.

RATIONALE

PAH emissions from atmospheric sources are in a category of phenomena that economists have labeled "negative externalities" or "negative spillovers." The designations imply that people are taking actions (e.g., producing coke, driving vehicles, and burning refuse) that generate, as byproducts or as incidental consequences, uncompensated costs imposed on other parties, outside of a market context; i.e., the PAH emissions produced incidentally by these activities ultimately have potentially unfavorable health consequences for others. In such situations, persons who are motivated largely by the prospect of private gain (or, in the case of firms, private profit) are unlikely to take corrective action. Without incentives for corrective action, too much of the activity will occur, and too little effort will be devoted to reducing the costs imposed on others.

An externality is an indication of a market failure;[3] i.e., even an otherwise properly functioning competitive economy will not achieve an optimal allocation of society's resources, because of the distortion introduced by the externality. In a private-enterprise economy, the source of the problem created by an externality can be traced to an ill-defined property right[8] (neither the emitters of PAHs nor those who are exposed have a well-defined property right to the ambient air and its cleanness) or to the difficulties of enforcing a property right. The latter difficulties are usually due to the "public-goods" aspects of the phenomena; e.g., because an improvement in air quality in a locality will be enjoyed by all, each individual has an incentive to let others make the necessary effort to enforce emissions reductions, and this incentive for "free riding" leads to too little (or no) action.

Externalities (especially those involving public-goods aspects) provide a case for possible public intervention in a private-enterprise economy. But whether, in practice, government intervention to correct an externality increases or decreases societal welfare is an empirical question.

LEVELS OF CONTROL

Once an externality has been identified and the decision has been made that some kind of corrective action is warranted, further decisions must be made on the extent of corrective action (e.g., the desired degree of reduction in PAH emissions or the amounts of PAHs that will still be allowed to be emitted) and on the specific tools that are to be used to implement the desired level of control. This section addresses the former issue, leaving the latter for the next section.

The control of an externality brings societal benefits: a reduction in the externality costs imposed on others. In the case of PAHs, reductions in PAH emissions that translate into reductions in human exposure to PAHs mean the avoidance of some premature deaths (frequently termed "the saving of lives") and the avoidance of PAH-induced illness. But the achievement of these benefits almost always involves societal costs: individuals and firms must be induced to change their behavior with respect to emissions, engage in less of their desired activities, and incur costs (use real resources) to reduce emissions.

Society's resources are scarce--in essence, society does not have limitless resources and cannot achieve all its desired objectives simultaneously, but must choose among them--and any level of externality control involves both societal benefits and societal costs; therefore, decisions concerning levels of control should focus on levels that best use society's scarce resources in trying to maximize societal welfare--i.e., society ought to aim for levels of control that provide the greatest margin of benefits relative to costs.

Two main analytic tools have been developed that can aid decision-makers in choosing the appropriate levels of control: cost-effectiveness analysis and cost-benefit analysis. Cost-effectiveness analysis is the more limited of the two. It takes, as a given, a specific societal goal (objective)--e.g., a reduction in emissions by X tons of a specific pollutant or the incurring of only up to Y dollars for the reduction of emissions from a specific source of that pollutant. The principle of cost-effectiveness requires a search to identify the least costly way of achieving a reduction in pollutant emissions. If all sources of the pollutant have equal environmental consequences, then the emission source with the lowest marginal (incremental) cost of control should be chosen. For example, if one source has a marginal control cost of $500/ton and another a marginal cost of $3,000/ton, the first should be chosen over the second. The choice of the first will mean that the achievement of emission reduction by X tons will require less resources, or the expenditure of Y dollars will achieve a greater reduction. The formal principle is that, in achieving the goal, the marginal costs of control from all sources ought to be equated. If this principle is violated, then the cost of achieving a given level of overall control could be reduced (or the level of overall control achieved at given costs could be increased) by increasing the stringency of control from the low-marginal-cost sources and decreasing the stringency of control from the high-marginal-cost sources.

Cost-effectiveness analysis can be a useful tool for improving the efficiency of individual programs and for comparing the effectiveness of similar programs. But cost-effectiveness analysis cannot be used to answer the ultimate policy questions: "Should X tons or 10X tons of pollutant-emission reduction be the appropriate societal goal?" "Should a cost of Y dollars or 20Y dollars be incurred to achieve emission reduction?" But cost-benefit analysis can provide an analytic basis for making these decisions.

There are only a few primary steps in a cost-benefit analysis. The societal benefits and societal costs should be estimated and converted into dollar equivalents (if they are not already in dollars). An interest rate (discount rate) must be used to convert future benefits and costs into present-value equivalents. The projects (or alternative versions of a project, e.g., alternative levels of stringency of required emission reductions) with the highest margins of benefits relative to costs should be the ones chosen. An equivalent principle is that, in choosing among alternative versions of a project (say, alternative levels of emission control stringency), stringency should be adjusted until the marginal benefits of extra stringency are just equal to the marginal costs. The basic methods of cost-benefit analysis are, by now, standard;[13,35]

controversies remain, however, as to the interest rate that should be used for discounting,[26] whether the income-distribution consequences of projects should be considered explicitly in the analysis,[19] how to incorporate risk and uncertainty into the analysis, and how (and whether) to place dollar values on nonmarket items and concepts.

In this last category, a frequent question that arises in the context of cost-benefit analysis applied to projects or programs that have mortality or morbidity consequences (e.g., many pollutant-emission control programs) is how (and whether) to evaluate the benefits of mortality or morbidity reduction. Claims that "a life is priceless" and that "one cannot put a value on a life or on pain and suffering" are often heard. A logical implication of these claims seems to be that cost-benefit analysis is useless in such instances--that for such projects, so long as any mortality reduction ("lives to be saved") or morbidity reduction (reduction in "pain and suffering") can be achieved, a project or program should be pursued (or extra stringency pursued), regardless of costs.

This approach to the benefits of reductions in mortality or morbidity does not provide a useful guide for making societal decisions, because the opportunities for achieving reductions in mortality and morbidity are virtually limitless. Additional resources devoted to medical research, medical care, accident prevention, and pollution reduction are likely to yield reductions (albeit possibly small) in mortality and morbidity. Society could use up its entire gross national product by devoting ever-increasing amounts of resources to the pursuit of such reductions. But, in fact, we do not. Through our societal decision-making processes, at some point we desist. For example, in the wake of the Arab oil embargo of 1973, the Congress enacted a law imposing a national highway speed limit of 55 mph. The major goal of the legislation was to reduce American gasoline consumption, but it was soon learned that the 55-mph speed limit had the beneficial side effect of reducing highway mortality. There have been no efforts to reduce the speed limit to, say, 45 mph, although such a reduction would clearly reduce highway mortality even more. Similarly, society does not build pedestrian underpasses for every busy urban intersection and does not station ambulances near those intersections, despite the reductions in mortality and morbidity that would be achieved. In effect, society has decided that the extra mortality and morbidity reductions are not worth the resources (costs) that would have to be devoted to achieving them; lines have been drawn.

Drawing these lines has been a largely implicit process; drawing them explicitly apparently makes many people uneasy. They are reluctant to put a value on mortality or morbidity reductions. But a society that wishes to achieve the best that it can from its scarce resources must understand the uses to which those resources are put and the tradeoffs (the "opportunity costs") involved. A society may well have multiple goals. Nevertheless, an understanding of the tradeoffs is important in pursuing them; and the use of explicit values for mortality and morbidity reductions is necessary for that understanding. Furthermore, the logic of cost-effectiveness argues for the consistency of these values across projects; otherwise, societal resources are allocated in an ineffective way, as apparently has been the case for actual projects and programs involving mortality and morbidity reductions.[2]

A good case can be made, then, for using explicit values for mortality and morbidity reductions. There are a number of candidates for establishing the value of mortality reduction (or, alternatively, "the value of a life"):

- The expected discounted future earnings of a person.
- The life insurance held by a person.
- The average (or some other summary measure) of the implicit values yielded by other, recent projects or programs involving mortality reduction.
- Compensation awarded in trials involving premature deaths.
- Estimates of the value that people, in their day-to-day behavior, place on incurring or avoiding risks of premature death.

For the purposes of deciding on the appropriate levels of pollution control, Bailey[2] and Freeman[16] (Chapter 4) have reviewed and criticized these measures. The last measure (risk valuation) is most consistent with the market valuations that are the other components of cost-effectiveness and cost-benefit analyses. An important point here is that pollution-reduction programs (and accident-reduction programs) do not have a knowable effect on specific persons' lives; they do not involve before-the-fact specific deaths. Instead, if they are effective at all, they reduce the probabilities or risks of the premature death of exposed persons. After the fact, this reduction in risk must mean a reduction in premature deaths; but before the fact, the programs can be evaluated only in terms of risk.

Because the affected persons benefit from the reduction in risk and because virtually all people expose themselves to risks in their day-to-day behavior (whether they acknowledge it or not), the benefit of the risk reduction should be roughly comparable with the value of the risks that they incur or avoid (at the margin) in their day-to-day behavior. In essence, if they are asked, "What would you be willing to pay in return for a reduction in risk?" or "What would you need to receive to compensate you for an increase in risk?" their responses should be roughly consistent with their private behavior. In a market economy, the prices of goods and services reflect (at the margin) a willingness to pay for those goods and services. Public projects, to maximize the societal value that can be achieved from society's resources, should also use willingness-to-pay measures for valuation purposes wherever possible. Accordingly, the risk-valuation approach is consistent for assessing pollution-reduction programs.

There are no specific markets in the private sector where one could directly observe a person's willingness to pay for risk reduction. But people do choose to incur or avoid risk, gaining or giving up other things in return, in most aspects of their lives: They choose jobs that have higher or lower risks of accidental death or injury, in return for explicit or indirect wage premiums;[36] they choose to use or not to use seatbelts in automobiles, trading off time and convenience against reduced risk of death or injury in the event of a crash;[4] they choose to live in neighborhoods with higher or lower air-pollutant concentrations, trading off housing costs against the extra risks of mortality or morbidity from the pollutants;[30] and so on. Economists have been able to provide models of individual behavior and, with actual data and econometric estimation techniques, estimate the implicit value that people have placed on the risks that they have incurred or avoided. For example, to estimate the wage premium that accompanies extra risk, a researcher could collect a sample of wage rates for various occupations, the actuarial data on accidental deaths for those occupations, and data on the various influences on wage rates (e.g., degree of unionization, amount of education, extent of experience). The econometric techniques allow the researcher to control for the other influences and thus to infer the implicit wage premium that accompanies extra risk.

Placing a value on reducing the risk of death is very difficult and controversial. Different values can be assigned. However, the values discussed here fairly represent the research that has been done in this field, and they provide a useful reference guide for decision-making with regard to pollution control.

Studies of the value of risk do not yield identical estimates, but, as Bailey[2] showed, they can be grouped (after appropriate adjustments and corrections) into a range of $170-715 (in 1978 dollars) in annual payment per 0.001 (i.e., 0.1%) additional annual risk of death. A study by Portney[30] yielded an additional estimate that is in the middle of this range. Freeman[16] argued that the most likely value is $1,000 (in 1978 dollars) per 0.001 additional risk. This same figure was used in the NRC study (pp. 244-245) of the costs of removing chloroform and other trihalomethanes from drinking water.[28]

Some problems of using these studies and the estimates they yield for evaluating public pollution-control programs should be noted. First, as with the use of any econometric model, one needs to be satisfied that the model has been properly specified and all important influences properly accounted for. Second, the models assume that the persons involved were aware of the risks they were incurring or avoiding. Third, use of the models' estimates for public-policy purposes assumes that the persons in the sample (and hence the estimates of the value of risk) are typical of the general population. If a wage study included only or mostly high-risk occupations, the resulting estimate of the value of risk might be an underestimate of the value that applies to most of the population, since persons with less fear of risk would likely gravitate toward high-risk occupations or housing locations--i.e., self-selection might bias the results. Fourth, people may feel differently about (value differently) risks over which they have more control (e.g., job choice) and risks over which they have less control (e.g., the general level of pollution in the air they breathe). Finally, even if the models' estimates are representative of the general population's valuation of risk, individual persons will have different values of risk and hence different perceptions of, say, the concentrations for which a pollution-control program should aim. Within a locality, however, all persons will have to be exposed to roughly the same pollutant concentrations.

The last problem is an unresolvable dilemma that is inherent in the public-goods aspects of most pollution problems, which cause them to be the proper concern of nonindividualistic, government action in the first place. This dilemma is present for all public goods (e.g., national defense and local police protection) that people consume generally automatically and equally as part of a community. As Samuelson[31] has demonstrated, the proper procedure for deciding on the appropriate level of a public-goods project is to sum the valuations of all affected persons and extend the project to the point at which the sum of the marginal valuations (benefits) equals the marginal cost of the extension--exactly the criterion stated in the discussion of cost-benefit analysis.

Despite the possible problems, the range of estimates yielded by the risk-valuation studies does appear to be reasonable when compared with the income of a typical family and the safety-related expenditures it would find worthwhile.[2]

One aspect of the risk-valuation estimates is worth emphasizing. If one finds that people appear to be willing to pay $500 per year each to avoid a 0.001 risk of death in a given year, the proper use of this estimate is as follows: Suppose a government pollution-control program can reduce the risk of death in a community of 1 million by a factor of 0.001. Then, because each person, on average, should be willing to value this improvement at about $500 per year, the 1 million people in the community should be willing to pay about $500 million per year for these benefits, and this aggregate value could be compared with the anticipated cost of the program. In essence, the aggregate cost of the benefit is estimated by multiplying the typical person's valuation of the risk reduction by the number of persons involved (reduction in risk per person).

In contrast, the value of risk is sometimes extrapolated to a value of avoiding (or, in reality, delaying) a death or "the value of [extending] a life"; i.e., the $500 per 0.001 risk would be extrapolated to $500,000 as the value of avoiding a death. It is true that, if the government implements the hypothetical program just mentioned, there will be 1,000 fewer deaths per year; and, because the program was valued at $500 million per year, this implies a value of $500,000 per avoided death. Furthermore, for some purposes, it is sometimes convenient to speak or write in terms of "the value of a life" (or the value of a statistical life). But there is nothing in the statistical or conceptual procedures that leads to the conclusion that any person would, could, or should pay $500,000 to avoid a certain death. Rather, before the fact, the government project promises a change in risk, not a change in the certainty of death for any person. People behave toward and implicitly value risk in their everyday life, so risk valuation is the consistent conceptual procedure to use.

The discussion thus far has focused entirely on valuing mortality changes. In principle, the same procedures could be applied to valuing changes in morbidity--i.e, willingness-to-pay measures could be inferred from persons' behavior. There do not appear to be any studies that have tried to generate such estimates. Instead, estimates of the medical costs and lost productivity related to illness and accidents are usually used to estimate these societal costs (and hence the societal benefits from their reduction). These estimates may not be too far away from what the appropriate willingness-to-pay measures, if they existed, would indicate, except that the former probably underestimate the latter by excluding the value of avoiding pain and suffering.

Finally, the limitations of cost-effectiveness and cost-benefit analysis must be acknowledged. Knowledge about costs and benefits is never perfect; in some cases, it may be quite imperfect. Risks and uncertainties often pervade analyses. Society has multiple goals. But, in the end, society's resources have to be allocated, and those resources are scarce and have alternative uses. Cost-effectiveness and cost-benefit analysis, imperfect though they may be, can be aids to effective societal decision-making.

IMPLEMENTATION

Regardless of the target level of control desired, a number of choices with respect to the implementation of an emission-control program are possible. A useful dichotomy is provided by the division between fiat methods (frequently called "command and control") and methods that rely on the use of economic incentives.

At one extreme, after a desired reduction in emissions (or a desired level of remaining emissions) has been ascertained, a central regulatory control agency can attempt to specify to each emitter (or class of emitters) the reduction or allowable emissions that will be required. If the agency wished to minimize the societal cost of achieving the emission reduction, it would try to have complete information about the total and marginal cost schedules for each of the various emitters and allocate reduction or emission appropriately, following the precepts of cost-effectiveness analysis.

At the other extreme, the agency could set an effluent fee that would require an emitter to pay a specified amount per unit of the pollutant that was emitted. In the presence of rising marginal costs of control, emitters would find it worthwhile to reduce emissions to the point at which the marginal cost per unit of pollutant reduction was equal to the effluent fee. The same knowledge of cost schedules assumed above would allow the agency to set an effluent fee that would achieve the same reductions as those achieved by the fiat method.

As the previous paragraphs indicated, under conditions of complete certainty, the two methods can achieve the same outcome. But knowledge about the costs of control is rarely complete. With incomplete knowledge, the control agency is likely to make socially costly mistakes by improperly assigning excessive emission reductions to emitters with high marginal costs of control. The effluent-fee system has an important advantage in this respect, in that it allows the high-cost and low-cost emitters to sort themselves out and achieve the lowest overall cost of control through their own behavior. Incomplete knowledge of costs may also lead to effluent-fee schedules that are too high or too low, with consequent emission reductions that are off target. But the schedules can be readjusted by continuing to observe emissions; incorrect assignments under the fiat method may never be corrected, because correct cost information is not automatically revealed.

An alleged advantage of the fiat method is its apparent certainty of outcome. Emitters will be told to reduce their emissions by a specified amount, and that reduction "will" be achieved. The effluent-fee method appears to be more indirect; one has to rely on the cost-reduction consciousness of firms and individuals to recognize that reducing emissions (up to a point) is less costly than paying effluent fees. But experience with pollution-control programs has shown that even the expected certainty of the fiat method often does not materialize.[27,44] Many emission-control programs are intended to be "technology-forcing"; they try to set emission standards that are beyond the economical range of current technology, thus attempting to force the development of advanced technology. The ostensible sanctions for failure to meet emission standards are usually severe fines or closure of offending companies. But if the technology appears not to be available, the sanctions are not credible or enforceable. Furthermore, regulators may have difficulty in ascertaining whether the necessary technology is or is not available or economical or whether a good-faith effort has been made to develop the needed technology.

As a consequence of these uncertainties, the emitters (especially in an industry with a relatively small number of large firms) have an incentive to slow down their own technology development. Thus, the apparent certainty of success of the fiat programs is not necessarily reflected in actual practice, as the delays in the implementation of many pollution-control programs have revealed.

Even if the sanctions behind them are thought to be credible, fiat methods can lead to the development of inefficient techniques. Technologies that are low in cost but that may fall short of the standards are unlikely to be pursued; technologies that can, at low cost, reduce emissions beyond the point set by the standards will be pursued only to the point set by the standards; technologies that are expected to be low in cost but have an uncertain likelihood of probability of success will be discarded in favor of high-cost, more certain technologies. An effluent-fee system would not have these inefficiency properties.

Another method of control that retains most of the incentive properties of effluent fees, but also has some of the possible quantitative certainty of a fiat system, is a system of marketable emission permits.[12] Under this system, the central regulatory agency sets a target of maximal total emissions of a given pollutant. It then creates a set of permits equal to this total. The permits are, in essence, a property right in a given amount of emissions. No one is allowed to emit without a permit; thus, each emitter must control emissions down to the point for which permits have been received. The agency could auction off the permits to the highest bidder (thus lodging the property right in clean air initially with the government), or it could initially assign the permits among emitters, or even among the population generally, in some manner (thus initially assigning the property rights in the manner chosen). If the permits are auctioned or can be traded, emitters will again sort themselves into an efficient, least-cost pattern, with low-cost emitters choosing to control emissions more and buying relatively fewer permits and high-cost emitters doing the opposite.

It is clear that, with appropriately chosen targets (costs and emissions), an effluent-fee system and a marketable-permit system can achieve the same outcome with comparable incentive effects. One difference between them is that the effluent-fee system always implicitly lodges the property right with the government, whereas the marketable-permit system may lodge the property right with the government (if the auction method is used) or in the private sector (if some assignment scheme is used). Another difference is in the identity of the group that bears the risk in the event of uncertainty about or variation in emitters' marginal-cost schedules.[32] In an effluent-fee system, variation in marginal-cost schedules will mean that variation can be expected in the quantities of emissions; thus, the risk is borne by those who are exposed to the emissions. In a marketable-permit system, variation in marginal-cost schedules will mean variation in the prices paid for the permits; the risk is borne by the emitters. The choice between the two systems on these grounds should be determined by examining the societal costs of lodging the risk with one group or the other. If, for example, the health consequences of small variations in emissions could be severe, a marketable-permit scheme would be preferred; if, however, the health consequences of small variations in emissions are not severe and the price variance of permits would cause firms to take relatively costly offsetting actions, the effluent-fee system would be preferred.

Even within the context of a fiat system, there are measures that increase the scope of economic incentives and efficiency. For stationary-source emissions, a "bubble" strategy that allows individual firms to trade off pollutant emission from different sources (e.g., different smokestacks) at the same geographic location provides the possibility of reducing the cost of controlling emission by a given amount.[23,25] In essence, an individual firm can exchange emission permits for its emission sources within the firm at the same location; this is a half-way step to a full marketable-permit system, which would allow firms to trade permits among different firms. Similarly, for motor vehicles, a fleet-averaging policy that would allow a manufacturer to satisfy emission standards if the sales-weighted average of its vehicles were at or below the standard, rather than every vehicle's being required to meet the standard, would allow the manufacturer to trade off low-cost ways (e.g., smaller vehicles) of meeting the standard against high-cost ways (e.g., larger vehicles) (White,[43] Chapter 7). Allowing vehicle manufacturers to trade (or even to "bank" for future use) any margin between allowed and actual emissions would convert a fleet-averaging scheme to a form of marketable-permit scheme. It should be noted, however, that fleet averaging, even with trading, is not identical with the standard marketable-permit scheme. The latter sets a limit on the overall amount of emissions, whereas the former sets a limit on the average per vehicle, but does not set a limit on the number of vehicles that can be sold. There are some circumstances in which a tightening of the standards in a fleet-averaging scheme could lead, perversely, to an increase in total emission.[22,44]

Overall, if regulatory schemes to control PAHs are put into effect, it appears desirable that the implementation tools chosen emphasize economic incentives and efficiency, regardless of the control levels that are selected as targets.

OBTAINING A BENCHMARK

A convenient way to start is to try to determine a societal value to place on a reduction by 1 ton/yr in PAH emissions from at least one important category of sources. If this benchmark figure can be established, the assessment of the likely costs and benefits of controlling PAHs from other categories of sources will be easier. The data from the analyses of Chapters 1, 2, and 3 and Appendix C, plus the risk valuation discussed earlier in this appendix, provide the basis for such a benchmark calculation. Again, we use BaP as a representative of PAHs. Thus, although emissions and concentrations are expressed in terms of BaP, they really represent a far larger "soup" of PAHs for which BaP is, in essence, the "tracer," or surrogate. Differences in particle size or other factors that might affect respirability or bioavailability are largely ignored. Linearity is assumed in most models.

The information in Chapter 1 shows that in 1979 the amount of BaP emitted into the atmosphere from urban road motor vehicles was sufficient to cause an urban commuter to inhale a calculated dose of up to 20.1 ng of BaP in the course of 24 h. By the year 2000, it is estimated that the same commuter would inhale only 9.1 ng/d. These estimates are based on inhalation rates of 15 m^3/d. Thus, for 1979, the average concentration of BaP from motor-vehicle emission in the air breathed by the "worst-case" person was 1.34 ng/m^3--i.e., (20.1 ng/d)/(15 m^3/d)--and for 2000 it would be 0.61 ng/m^3. In the first year, 13.46 metric tons of BaP was estimated to be emitted by motor vehicles, and in 2000, 10.14 metric tons. If we adopt a rough linear model relating the gross BaP emissions per year to average concentration, we estimate that 1 ton = 0.1 ng/m^3 for the first year and 1 ton = 0.06 ng/m^3 for the second year. In later calculations, the first figure is used as a conservative estimate. Thus, it is assumed that a reduction of 1 ton of BaP emissions per year from motor vehicles is likely to reduce average urban BaP ambient concentrations by 0.1 ng/m^3 for that same year.

Next, this change in BaP concentration should be converted to an equivalent mortality risk. In Appendix C, it is estimated that exposure to BaP at an average of 1 ng/m^3 for an entire lifetime leads to a cumulative excess risk of lung cancer by the age of 70 of 0.02-0.06%. Again, the latter figure is used as a conservative estimate. Thus, if it is also assumed that a ton of BaP (representing a larger quantity of PAHs) from motor vehicles has the same health consequences as a ton of BaP (also representing a larger quantity of PAHs) from another atmospheric source, then breathing BaP from motor vehicles at an average of 0.1 ng/m^3 would have a cumulative excess risk of 0.006%, or 0.6×10^{-4}. This would be the same risk generated by 1 ton of BaP emission per year for 70 yr. But it is necessary to determine the risk generated by 1 ton of BaP in 1 yr. As an approximation, this extra risk can be "smeared" equally over all 70 yr. Thus, the extra risk of premature death per year is 0.00009%, or a 0.9×10^{-6} probability of a premature death in each year. (Although the original 0.006% is a cumulative risk to age 70, with the risk of premature death in each year rising exponentially, the absolute numbers are small enough so that "smearing" equally makes little difference in the results.)

Finally, a value can be put on this probability. The range of the annual value of avoiding a 0.001 annual probability of a premature death, reported above, was $170-1,000 (in 1978 dollars). To be conservative, the upper limit will be used and translated into a 1982 dollar figure of about $1,500 per 0.001 risk. This figure, then, indicates that the reduction in annual risk of 0.9×10^{-6} would be worth about $1.35/person. It should be recalled that the atmospheric-concentration data apply to urban areas. Approximately 75% of the U.S. population of 225 million live in urban areas, or about 170 million. Accordingly, these calculations indicate that the reduction in BaP emissions by 1 ton in 1 yr, which would lower urban ambient concentrations of BaP by about 0.1 ng/m^3 and thus lower the annual risk of death per person exposed by 0.9×10^{-6}, would be worth about $225 million in the aggregate, or $225,000/kg, $225/g, or $0.22/mg of BaP.

It should be noted again that BaP is being used here as a tracer to represent a larger collection of PAHs and that the $0.22/mg of BaP really represents the value of controlling this larger "soup" of PAHs that has a potency that can be measured and represented by 1 mg of BaP. Also, the risk valuation applies only to the lung-cancer consequences of exposure to PAHs; other possible mortality and morbidity consequences of exposure have been ignored.

Furthermore, it should be emphasized that each of the key components of the value estimate is an estimate that has a substantial range of uncertainty. The risk of death associated with breathing BaP at a given concentration has an uncertainty range of approximately a factor of 3; the societal value of avoiding a premature death has a range of approximately a factor of 6; and the likely atmospheric concentration from a ton of BaP has a range of 1.5. Because these estimates are used multiplicatively, the overall range of uncertainty on the final value estimate is approximately a factor of 25. For the present analysis, in each case the most conservative estimate of each component--the figure that would indicate the greatest societal benefit from controlling PAHs--was used. Alternative methods would have been to use the most likely value for each component and to carry the range throughout. But information for choosing the most likely values is not available; and, as noted, carrying the range throughout leads to an uncertainty range of a factor of 25 downward from the estimate of a societal value of $225 million per ton of BaP removed. Thus, at the other end of the range, those who prefer to be less conservative could use a value as low as $9 million per ton of BaP removed. In matters of public decision-making concerning the societal value of actions that involve avoiding premature deaths--a highly controversial subject--a conservative approach seems warranted. Accordingly, the figure of $225 per ton is used for the remainder of this analysis.

CONTROL OF PAH EMISSIONS FROM VARIOUS SOURCES

Although PAHs are the product of virtually every burning process, it makes sense to focus on the quantitatively important sources. Chapters 1-4 and other studies[15] indicate that the following sources are important (not necessarily in order of quantitative importance):

- Road motor vehicles.
- Other mobile sources (e.g., trains, planes, and ships).
- Fireplaces.
- Wood-burning stoves.
- Residential coal-fired heating.
- Industrial coal-fired boilers.
- Coke production.
- Industrial-commercial incinerators.
- Agricultural open burning.
- Land-clearing waste burning.
- Prescribed burning of underbrush in forests.
- Forest and prairie fires.
- Structural fires.
- Coal-refuse fires.
- Volcanoes.

The control of PAH emissions from some of these sources can be ruled out, because they cannot be controlled (such as volcanoes). In principle, reductions in PAH emissions could probably be achieved by applying more resources to controlling forest fires, structural fires, and coal-refuse fires (largely in abandoned coal mines). But the other societal costs from these sources probably bulk so large in comparison with their PAH-related costs that greatly increased efforts to combat these fires could not be justified solely on the basis of their PAH emissions.

Other sources may offer some promise of worthwhile control. The discussion here begins with road motor vehicles, because the data on them are best, and then examines stationary sources. In each case, only the reductions in PAH emissions are valued and compared with the costs of the reductions. In many instances, the effort to reduce PAH emissions will reduce other harmful pollutants as well (e.g., particulates in general). The reductions in these other pollutants may have additional societal value; but that value is not calculated or considered here. Also, in some instances, efforts to reduce PAH emissions may increase the emissions of other pollutants. These additional effects are ignored. Thus, the discussion here focuses on whether reductions in PAH emissions, valued alone, justify (or come reasonably close to justifying) control efforts.

ROAD MOTOR VEHICLES

As noted earlier, gasoline-powered vehicles without exhaust catalytic converters (i.e., all pre-1975 cars and light-duty trucks and all heavy-duty trucks and buses of any vintage) and diesel cars, trucks, and buses constitute the major sources of PAH emission from road motor vehicles. An additional category of "problem" vehicles would include cars and light-duty trucks of the 1975 and later model years that have emission control systems that are no longer functioning properly. The categories of gasoline and diesel vehicles are addressed separately. Unless otherwise indicated, urban-rural driving distinctions are ignored, and emission reductions in rural areas are valued at the same rate as urban reductions.

Gasoline Vehicles

At the beginning, it is useful to establish a relationship between total hydrocarbon (HC) emission per mile and BaP emissions per mile for gasoline-powered vehicles. The data in Chapters 1-3 and in Williams and Swarin[45] indicate an approximate relationship--HC at 1 g/mi = BaP at 2 μg/mi--that is used in the discussion that follows.

Once motor vehicles are manufactured and on the road, there are three major ways to reduce emissions (including that of PAHs) from them: retrofitting them with further controls, inducing better maintenance and slower deterioration of their control systems, and inducing owners to junk them in favor of newer vehicles.

Retrofitting does not seem to be a practical method; it would probably achieve only modest emission reductions, and it is quite unpopular. Only one state (California) has a program for requiring retrofitting of older cars, and that program applies only when cars change owners.[21]

Gruenspecht[18] has analyzed the prospect of providing subsidies to owners of older cars to junk them in favor of newer ones and finds it a worthwhile strategy, compared with the costs of the tighter standards imposed for the 1981 and later model cars. The societal benefits from PAH reductions, not included by Gruenspecht, would add to his results. An older car that emitted HC at 4 g/mi (and hence BaP at 8 μg/mi) more than a new car and that was expected to last for another 40,000 mi of operation would emit 320 mg of "extra" BaP during this period. The risk-valuation calculations of this chapter have shown that a reduction of this amount of BaP would be worth $70 to society. Thus, the bonus or subsidy paid to owners of old cars to induce them to junk the vehicles could be increased by this amount, to induce yet more turnover of the fleet.

Better maintenance of emission control systems can be induced by inspection and maintenance (I&M) programs by states and locales. White[44] examined these programs and concluded that they could be worthwhile under some circumstances, especially if linked to safety-inspection programs. The societal value of the reduction in emissions of all pollutants achieved by such programs was estimated to be $23/vehicle, with $5 of this coming from the 5 kg of HC reduction per year per vehicle that would be achieved. The values were based on the comparative costs of achieving the equivalent reductions in emissions from other sources. To the extent that vehicle HC emissions contained appreciably more BaP than the HC emissions from other sources, this might raise the societal value of the reduction. The limit of this increase would be $2/vehicle [(10 mg)($0.22) ≅ $2]. This figure is well within the margin of error of the original calculations and hence does not appear to make I&M programs appreciably more attractive than they otherwise would be.

One other source of improved maintenance can be examined. The U.S. Environmental Protection Agency (EPA) reported that 5-10% of 1975 and later cars have used leaded fuel, which, after five or six tankfuls, permanently poisons and renders useless the catalytic converters on these cars.[38] If only unleaded gasoline were sold, this poisoning would not occur. The societal value, from the perspective of PAH emissions, of this change to the production and sale of only unleaded gasoline can be calculated.

The effect on HC (and hence PAH) emissions of the loss of effectiveness of the converter depends on the way the manufacturer has tuned the remainder of the control system. If we use a change in HC of 2 g/mi as a likely estimate, this implies additional emissions of BaP at 4 μg/mi. Suppose that 10% of the catalytic-converter fleet (cars and light-duty trucks) has poisoned catalysts and that this fleet accounts for 70% of the 140×10^{10} mi driven annually by gasoline vehicles. Then the extra BaP emissions from the poisoned-catalyst vehicles come to 392 kg of BaP per year ($4\ \mu g/mi \times 0.1 \times 0.7 \times 140 \times 10^{10}$ mi/yr). The risk-valuation procedure indicates that the elimination of these emissions would be worth about $86 million. (This counts reductions in both urban and rural BaP emissions as worth $0.22/mg; if only urban-emission reductions are considered valuable, because rural emissions are more dispersed and are relatively less harmful, the value would be only 60% as great, or $52 million.)

To achieve this gain, the 40 x 10^9 gal of leaded gasoline would have to be replaced by unleaded. A conservative estimate of the cost of this conversion would be $0.05/gal--a low estimate of the difference in retail price between leaded and unleaded gasoline. (There appear to be no good reasons why the difference in retail price should not fully reflect the difference in the complete cost of production, distribution, and sale of the two types of gasoline.) Thus, conservatively, the conversion would cost at least $2 billion/yr. Because unleaded gasoline tends to have a higher aromatic-compound content than leaded and a higher aromatic-compound content tends to yield greater PAH emissions, the conversion would tend to yield greater PAH emissions from older vehicles and from all heavy-duty trucks--but lead emissions would decrease. In sum, the benefits from PAH reductions alone appear to be far smaller than the costs of converting the U.S. gasoline supply entirely to unleaded.

The HC emission standards for heavy-duty gasoline trucks currently limit HC emissions to the equivalent of about 5 g/mi, or about 0.5 ton over the life of a truck. The heavy-duty truck regulations likely to be promulgated for 1984 and after will decrease this to about 0.23 ton of HC over the life of the vehicle.[42] Further tightening of standards (as originally promulgated for 1984) could decrease lifetime HC emissions by an additional 0.08 ton; this would yield a decrease of roughly 0.16 g of BaP. The risk-valuation method indicates that this decrease would be worth $35. EPA has estimated that the hardware (largely, a catalytic converter) for this further tightening of a standard would cost about $300; some manufacturers have indicated higher values. In any event, the value of the reduction in the amount of BaP is unlikely to make an appreciable difference in assessing the value of the change in HC over the life of the vehicle.

In sum, for gasoline vehicles, the likely benefits from reductions in PAH emissions appear to fall far short of the costs of the measures that would be necessary to achieve them.

Diesel Vehicles

As noted in Chapter 1, diesel vehicles are important sources of PAHs, with most of these compounds adsorbed on the surface of carbon particles. Also given in Chapter 1 are estimates of BaP emissions of 13 μg/mi and 54 μg/mi for light-duty and heavy-duty diesel vehicles, respectively, and they are used initially here.

Reductions in particulate emissions from light-duty diesels have thus far been achieved through engine modifications; because no regulations have been promulgated for particulate emissions from heavy-duty diesels, it is unlikely that any reductions in emissions from these vehicles have occurred.

Further reductions in emissions from light-duty diesels appear to be possible from two sources: trap-oxidizers in the exhaust and fuel modifications.

Assume that a trap-oxidizer, in reducing particulates, reduces PAH emissions by a comparable percentage. EPA regulations currently mandate a standard of a particulate maximum of 0.6 g/mi for 1982-1984 light-duty diesels. For 1985 and after, current EPA regulations require standards of 0.2 g/mi for diesel cars and 0.26 g/mi for diesel light-duty trucks. It appears unlikely that the required trap-oxidizer technology will be available, and these regulations are likely to be modified. Nevertheless, because the trap-oxidizer technology has been pursued in the context of the 0.2-g/mi standard, it is useful to analyze emissions in the same context.

The 0.2-g/mi standard would imply a roughly two-thirds reduction in particulate emissions. If BaP emissions fall by the same proportional amount, this implies a reduction of 8 μg/mi. Over the typical life of the vehicle (100,000 mi), there would be 0.8 g less BaP emitted from the vehicle. The risk-valuation procedures indicate that this reduction would be worth $176. (In principle, a discount rate should be used for benefits after the first year. Because of the pattern of use of these vehicles, a real discount rate of 3% would reduce the net present value of the benefits by only 10%.) General Motors currently estimates that a trap-oxidizer, if it is made practicable, will cost around $500;[17] EPA has estimated the cost as appreciably lower.[40] There may also be fuel-economy penalties and driver-satisfaction costs. The societal value of the reduction in PAH emission alone would offset only a modest fraction of the cost of control.

As to heavy-duty diesel trucks, in late 1980 EPA proposed a set of regulations that would have reduced particulate emissions from heavy-duty diesels built in 1986 and later to one-third the emissions from unregulated vehicles.[39] EPA has taken no further action to make these rules final, and, because they too required trap-oxidizers, it appears likely that they will be modified. But they are useful as a benchmark. A two-thirds reduction in BaP emissions would mean a reduction of 36 μg/mi. Over the typical life of a heavy-duty diesel vehicle (475,000 mi), this implies a reduction in BaP emissions of 17.1 g. The valuation method indicates that this reduction, if it all occurred in urban areas, would be worth $3,760. (These estimates do not incorporate a discount rate.) Here, however, the rural-urban distinction cannot be ignored. The data in Chapter 1 indicate that only 20% of heavy-duty diesel mileage is likely to occur in urban areas. Thus, these estimates are upper limits of their likely societal value. If BaP emissions in rural areas were assumed to have little or no serious human-health consequence (i.e., it is transformed or otherwise removed in an ultimately harmless fashion before an appreciable number of people are exposed), then the societal value (based only on urban exposure) would be only $752. This last value is still relatively large. The reduction in PAH emissions from heavy-duty vehicles seems to be societally important (in essence, because of the relatively heavy emissions from and the high mileage accumulated by these vehicles). Even if the large reductions attempted by the regulations proposed in late 1980 cannot be achieved, it appears that smaller reductions (which might be achieved through relatively low-cost engine modifications, analogous to those already achieved in light-duty diesels), although also promising smaller benefits, would be societally worthwhile on the basis of PAH emissions alone. In this respect, this appendix can echo the recommendation of the recent NRC study of light-duty diesels: "Regulate particulate exhaust from such large sources of emissions in road transport as heavy diesel trucks and buses; this may be more cost-effective than tightening the emission levels of diesel cars and light trucks."[29]

The substitution of No. 1 diesel fuel for the currently used No. 2 diesel fuel can reduce particulate emissions and PAH emissions.[5,6,20] The results of Hare and Baines[20] indicate that BaP emissions from light-duty vehicles may be reduced by about 25%. An experiment on Washington, D.C., buses suggests that the reduction might be even greater for heavy-duty vehicles.[24] The estimate of 25% is used here.

The BaP emissions from both light- and heavy-duty diesels is 320 μg/gal. A 25% reduction would mean a reduction of 80 μg/gal. The risk-valuation procedures place a value of $0.018 on this reduction. The current retail-price difference between the two fuels is around $0.08 per gallon. It does not appear that the benefit from the PAH reduction alone would exceed the costs of the substitution of No. 1 for No. 2 diesel fuel.

In sum, efforts to achieve engine modifications in heavy-duty vehicles appear to be the most cost-effective way to achieve net societal gains.

A few caveats should be mentioned with respect to the discussion of diesel vehicles. First, the diesel analyses assume that the PAH emissions from diesel vehicles, as represented by BaP, have the same health consequences per ton as the PAH emissions from other sources. But a recent NRC study[29] failed to find any definite association between diesel-exhaust emissions and carcinogenesis in humans, despite the presence in the exhaust of PAHs that are known to be carcinogenic in other animals. The study's authors suggested that there may be something special about the bioavailability of these compounds to humans when they are present in diesel exhaust. At the other extreme, however, the data in Appendix C indicate that the extracts from some diesel exhaust may be as much as 89 times more potent mutagenically when measured on a BaP-equivalent basis. The discussion in this section steers a middle course and assumes that the BaP in vehicle exhaust represents the same carcinogenic potential for humans as does the BaP from the sources covered in the review of epidemiologic studies in Appendix C.

Second, the results of Springer,[33] Hare and Baines,[20] and Williams and Swarin[45] provide estimates for light-duty diesel BaP emissions of around 3 μg/mi, with some vehicles achieving emissions below 1 μg/mi; these estimates should be compared with the figure of 13 μg/mi used in Chapter 1 and in this chapter. Thus, BaP emissions from light-duty diesel vehicles may be an order of magnitude lower than the figure used here, and the same qualification may apply to heavy-duty diesels.

OTHER TRANSPORTATION VEHICLES

Chapter 1 indicated that airplanes and ships are the major sources of BaP emissions in this category. Little other information appears to be available on emissions or possible avenues of control. Because most of these emissions occur outside urban areas, it is probably safe to neglect them in this analysis.

WOOD-BURNING STOVES

As the prices of other fuels have risen, burning wood for residential heating has become more popular. Wood stoves have 2 or 3 times the thermal efficiency of fireplaces and have become increasingly popular. It is estimated that a million wood-burning stoves were sold in 1979,[9] and sales have been increasing. Much of this wood-burning occurs in rural areas, but a substantial amount occurs in or affects urban areas. For example, Cooper _et al._[10] found that approximately 50% of the respirable particles in the ambient air of Portland, Oregon, in January 1978 came from residential wood combustion. Cooper also estimated that residential wood combustion emitted 1.4 tons of BaP in Portland's ambient air during 1978.[9]

It appears that wood-burning stoves emit BaP at about 2.5 mg/kg of wood burned.[9] Chapter 2 cited data that indicate that households burning wood as the primary source of heat each used an average of 5.6 metric tons of wood. It is likely that such a household used a wood stove. Thus, it would emit 14 g of BaP per year. (Thus, in terms of BaP emissions, one wood stove is the equivalent of over 100 diesel cars each emitting 13 μg/mi.) If we assume that emissions from a wood stove in an urban area have about the same effects on ambient BaP concentration as do vehicle emissions, we can use the valuation method to indicate that the complete elimination of these emissions would have a societal value of $3,080 _per year_.

Thus, it appears that the benefits from the control of PAH emissions from wood-burning stoves, especially in urban areas, are quite large. Unfortunately, research on emissions from wood-burning stoves is still at a relatively early stage. It appears that the design and structure of stoves can make some difference in PAH emissions.[14] Even more importantly, catalytic combustors (similar to the catalytic converters on cars) installed in the chimneys of wood stoves may be capable of reducing organic-compound emissions by up to 95%.[41] These control devices, if they become practicable, are expected to cost, installed in a new stove, around $125-150. It is unclear how durable they are, but even if they lasted only a year, it appears that their likely benefits greatly outweigh their likely costs.

It appears unrealistic to expect any feasible program for requiring retrofitting of existing wood stoves in residential use. But many current owners of wood stoves may be "environment-conscious" and might be prepared to retrofit their wood stoves voluntarily if retrofit devices were available and there were sufficient publicity. And a program to require (or induce) manufacturers of new wood stoves to incorporate changes in design or technology that would reduce PAH emissions appears to have great societal benefits and relatively small societal costs.

RESIDENTIAL FIREPLACES

Residential fireplaces are a large source of PAHs; they emit about one-third as much BaP as wood-burning stoves per kilogram of wood.[9]

It is unlikely that any emission-control program aimed at fireplaces would be feasible. It is unclear whether any technology is currently available for controlling emissions from fireplaces; even the catalytic combustors, which appear promising for wood stoves, are unlikely to be practicable for fireplaces, because the combustors require a higher temperature than most fireplace chimneys are likely to provide. Furthermore, any retrofitting program would be highly unlikely to be put into effect, and installation of any technologic device in new residences would deal with only a tiny fraction of the problem.

Nevertheless, because the aggregate amount of PAH emissions from fireplaces is large, and likely to grow, this appears to be a fruitful direction for research.

RESIDENTIAL COAL-FIRED HEATING

There appears to be little available information on this subject. Some residential heating units are capable of burning both wood and coal. Because the emissions from these units are potentially large, more information should be collected and research encouraged.

INDUSTRIAL COAL-FIRED BOILERS

Comparatively little appears to be known about the properties of these boilers. EPA still appears to be in the data-collecting stage with respect to these devices.[1,7] Because these boilers are still being manufactured and are in the hands of relatively larger and more sophisticated users (compared with households), control efforts would probably be feasible if the cost-benefit ratios were favorable. Retrofitting, inducement to use gas and oil, and improved design and technology (e.g., possibly catalytic combustors or precipitators) appear to be possible. Clearly, more research is necessary.

INDUSTRIAL-COMMERCIAL INCINERATORS

Municipal incinerators do not appear to be a serious source of PAH emission, but industrial-commercial incinerators are.[15] One possible reason is that municipal incinerators operate at higher, more efficient temperatures.

Unfortunately, little other information is available about these sources. Two control strategies seem to be possible. One would be to focus on the technology of industrial incineration itself--i.e., focus on retrofitting, improving the design of new devices, exploring the possibility of combustors or precipitators, etc. Again, one would want to make sure that the cost-benefit ratios were favorable before embarking on such an effort.

A second control strategy would be to require that industrial-commercial trash be hauled to municipal incinerators for burning. A rough estimate of the costs and benefits of such a strategy can be provided. If industrial-commercial incinerators have BaP emission rates of 120-570 µg/kg of refuse burned, burning a ton of refuse would yield 120-570 mg of BaP. If this occurred in urban areas and the emissions had a dispersion pattern similar to that of motor-vehicle emissions, the risk-valuation method would indicate that the complete elimination of these emissions would be worth $26-125. It appears that municipal incinerators have BaP emissions rates 2-3 orders of magnitude lower than industrial-commercial incineration rates.[15] Thus, the use of municipal incineration would mean the virtual elimination of the BaP emission.

In 1975, the cost of refuse collection was about $25/ton.[34] This figure should probably be doubled to bring it to 1982 dollars, for an estimate of $50/ton. This cost estimate is within the range of the likely benefits from the reduction in industrial-commercial incineration. Accordingly, efforts to reduce industrial-commercial incineration or the emissions from industrial-commercial incineration may be worthwhile and bear further investigation.

COKE-OVEN EMISSIONS

Coke ovens are well-known sources of PAH and BaP. Coke manufacturers are currently in the process of implementing emission reductions under EPA-supervised state implementation plans, consent decrees, and Occupational Safety and Health Administration plans. EPA has recently proposed further standards that would control emissions to a greater degree.[37] EPA estimates that its standards will reduce emissions by 880 tons of benzene-soluble organic compounds (BSOs) per year. EPA estimates the cost at $46 million/yr. The ratio of BaP emissions to BSO emissions appears to range from 1:500 for wet-coal charging to 1:133 for battery stacks.[15] This yields a range of 1.76-6.62 tons by which BaP emissions would be reduced by the proposed regulation. The risk-valuation procedure indicates that even the lower estimate is worth $387 million. This allows for substantial error in the estimates of costs and benefits that would nevertheless leave the proposed regulations cost-effective. Unfortunately, although EPA discussed yet more stringent regulations in its proposal, no cost figures were presented, so no evaluations can be made. The large margin between the likely benefits and the likely costs of control, however, indicates that further restrictions in coke-oven emissions could well be worthwhile (up to the point at which marginal costs equal marginal benefits).

PRESCRIBED BURNING

The burning of underbrush in forests appears to be a standard practice. It is claimed that such burning reduces the incidence of wildfires and achieves a substantial (5- to 10-fold) net reduction in particulate emissions.[11] If PAH emissions follow the same pattern, there appears to be little or no necessity for any corrective action.

AGRICULTURAL AND LAND-CLEARING WASTE BURNING

These are standard practices and take place in rural areas. The alternatives to burning appear to be collection and either central burning (at high, more efficient temperatures) or disposal in landfills. The BaP emissions from waste burning appear to be 190-430 μg/kg of waste burned.[15] This is roughly the same range as for industrial incinerators, and a similar analysis can be applied. It is not clear whether the cost per ton of rural collection of waste is higher or lower than the urban cost. Landfill disposal could add another $10/ton. Consequently, the same range of cost-benefit uncertainty that applied to industrial-commercial incineration appears to apply to rural waste burning--with the added element that these emissions are in rural areas and hence may be less socially damaging. Further study is warranted.

SUMMARY

This appendix has discussed the principles of public decision-making with respect to PAH emissions and has provided illustrative risk-valuation analyses to examine the societal costs and benefits of reducing PAH emissions from the major sources.

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